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IN RE APPLICATION OF: Giampiero DE LUCA
SERIAL NO.: NEW U.S. PCT APPLICATION
FILED: HEREWITH
INTERNATIONAL APPLICATION NO.: PCT/EP03/50303
INTERNATIONAL FILING DATE: July 10, 2003
FOR: USE OF COMPOUNDS FOR INCREASING SPERMATOZOA MOTILITY

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119
AND THE INTERNATIONAL CONVENTION

Commissioner for Patents
Alexandria, Virginia 22313

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
EPC	02100799.2	10 July 2002
EPC	02102876.6	23 December 2002

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP03/50303. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02102876.6

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1 (a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Use of compounds for increasing spermatozoa motility

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Use of compounds for increasing spermatozoa motility

Field of the invention

The invention relates to a process for the improvement of spermatozoa fertilization activity, in particular for the increase of spermatozoa motility by using a compound of formula (I). The invention further relates to the use of a compound of formula (I) in the treatment of infertility and assisted reproduction techniques as well as methods of use thereof, and to a medium for storage and/or transportation of spermatozoa comprising the use of a compound of formula (I).

Background of the invention

The infertility of a couple is defined as the inability of the woman to conceive after at least a year of regular unprotected sexual relations. Infertility may be caused by a multitude of factors, in which male factors play a fundamental role in around 40-50% of cases. Reduced male fertility is generally linked to alterations in seminal parameters such as morphology, motility and sperm count.

Various assisted reproduction techniques (ARTs) are proposed as treatment for infertility of the couple, in many cases making it possible to overcome the problem of both male and female factors. These methods, the choice of which depends on the type of diagnosis made, may involve the collection of male and female gametes (spermatozoa and oozytes). The further treatment varies according to the cause of the infertility. The gametes may be transferred directly into the Fallopian tube (GIFT= Gamete Intra Fallopian Transfer) or are brought into contact with each other in a test tube. If the latter leads to fertilization of the oozyte, the resulting zygote or embryo is transferred into the uterus (IVFET = In Vitro Fertilization and Embryo Transfer).

When infertility is due to male factor(s), parameters of the seminal liquid and in particular the count and motility of spermatozoa determine the choice of the particular assisted fertilization method used. In the most serious cases of male-factor infertility the spermatozoa count and/or their motility is very low. The fertilization activity of semen is usually assessed in a spermogram. According to WHO standards, which can be taken from the "WHO manual" (WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions, 4th edtition, Cambridge University Press 1999), semen are classified into the following groups:

- Normozoospermia: When all the spermatozoal parameters are normal together with normal seminal plasma ,WBCs (White blood cells) and no agglutination;
- Oligozoospermia: When sperm concentration is < 20 million/ml;
- Teratozoospermia: Fewer than 50% spermatozoa with forward progression (categories (a) and (b)) or fewer than 25% spermatozoa with category (a) movement;
- Asthenozoospermia: Fewer than 50% spermatozoa with normal morphology;
- Oligoasthenoteratozoospermia: Signifies disturbance of all the three variables (combination of only two prefixes may also be used);
- Azoospermia: No spermatozoa in the ejaculate.

Normal values of semen parameters have been issued by WHO that are generally used as reference. The fraction of motile sperm in semen is measured either by manual counting or using a computer assisted semen analysis (CASA) system. Motility is assessed at the time of semen liquefaction and after 1 and 3 hours to detect asthenozoospermia. Manual counting classifies sperm cells into 4 categories (immotile, locally motile, non linear and linear motile) using qualitative subjective criteria of selection. Many infertility centers now use CASA systems for objective measurements of sperm motion and positive correlations have been found between motion parameters such as the amplitude of lateral head displacement, curvilinear velocity, linearity and straight-line velocity and fertilization rates

in vitro but the threshold levels for these motion characteristics have not yet been established to meet a general consensus.

In case of severe male factor infertility, micro-assisted fertilization techniques can be used. Among these techniques, intracytoplasmatic sperm injection (ICSI) is the most common and has the highest percentage of success. However, the safety of the ICSI procedure for the health of the resulting conceptus or embryo is still matter of debate (*Nature Medicine* 5, 377-378 (1999) by Edwards RG). In addition, ICSI is far more expensive and more time consuming as compared to IVF.

Thus, the possibility to recover a higher number of spermatozoa showing a higher motility could allow several oligoasthenospermic men to enter IVF rather than ICSI programs.

Various methods have attempted at increasing the motility of the spermatozoa, like treatment of spermatozoa with pentoxyphylene, platelet activating factor or progesterone, for instance. However, the results obtained are variable and the responsiveness of the spermatozoa is not predictable.

Therefore, the finding of new methods and agents to improve sperm cell motility, leading to an improvement of the fertilization activity or fertilization rate, is highly desirable and urgently needed. These are objects of the invention to provide new methods and process to improve said sperm cell motility by using specific phosphatidylinositol-3-kinases inhibitors.

These phosphatidylinositol-3-kinases (PI3Ks) belong to a family of enzymes involved in signal transduction of tyrosine kinase receptors. Phosphatidylinositol-3-kinases, also called phosphoinositide-3-kinases (PI3Ks) generate lipids which are implicated in receptor-stimulated signalling and in the regulation of membrane traffic. Several distinct classes of PI3Ks have been identified that have been conserved throughout eukaryotic evolution. Potential signalling pathways downstream of PI3Ks have been elucidated and PI3K function is being characterized in several model organisms, as reviewed e.g. by Vanhaesebroeck et al.(*Trends Biochem. Sci.* 22 p.267-72 (1997)). PI3Ks are heterodimeric

enzymes present in various isoforms and composed of a catalytic subunit of 110 kDa, which is associated with a regulating subunit of 85 kDa.

In somatic cells phosphoinositide-3-kinases (PI3-kinases) are activated upon interaction with both receptor tyrosine kinases (RTK) and G-proteins resulting in the production of moieties involved in the inositol phospholipid signalling pathway. The enzyme is also present and active in human spermatozoa.

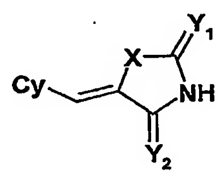
Several selective inhibitors of PI3Ks have been described. Wortmannin is one of the most well-known specific inhibitors. Wortmannin is a fungal metabolite derived from T. wortmanin (Kyowa Hakko Kohyo Co. Ltd.) or from P. fumiculosum (Sigma). Wortmannin and analogs thereof have already been described in patent literature (e.g. EP0635268 A1, EP0648492 A2 or EP0658343 A1). These compounds are known to be involved in the treatment of neoplasms, atherosclerosis, and bone disorders. Other phosphatidylinositol-3-kinase inhibitors are 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), and bioflavonoid quercetin for example described in Vlahos et al. in (*J. Biol. Chem.* **269**, p.5241-48 (1994)) and (*J. Immunol.* **154**, p.2413-22 (1995)).

The use of PI3K inhibitors in a process for the improvement of spermatozoa fertilization activity as well as for the preparation of a pharmaceutical composition in the treatment of infertility, particularly male infertility, has been disclosed by Applied Research Systems ARS Holding N.V. (WO 01/07021). In said patent, PI3K inhibitors are selected from the group consisting of 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), wortmannin, quercetin and derivatives and analogues thereof.

It has now been found in accordance with the invention that phosphatidylinositol-3-kinase inhibitors of formula (I) can improve the parameters determining sperm cell fertilization activity, in particular the sperm cell motility.

Summary of the invention

The invention therefore relates to a method of enhancing spermatozoa fertilization activity, in particular of increasing the motility of the spermatozoa, comprising the step of treating the spermatozoa by using a compound of the following formula (I)



(I)

wherein X, Y^1, Y^2 and Cy are defined in detail in the description below.

The invention further relates to spermatozoa in which the activity of the phosphatidylinositol-3 kinase is inhibited, as well as the use of a compound according to formula (I) for improving the fertilization rate in assisted reproduction techniques (ART). A third aspect of the invention concerns the use of a compound of formula (I) for the preparation of a pharmaceutical composition for the treatment of infertility, in particular male infertility. A fourth aspect of the present invention relates to methods of ART therapy comprising treating spermatozoa with a compound of formula (I). A fifth aspect of the invention relates to a medium for storage and/or transportation of spermatozoa containing a compound of formula (I).

Description of the invention

The following paragraphs provide definitions of the various chemical moieties that make up the compounds according to the invention and are intended to apply uniformly throughout the specification and claims unless an otherwise expressly set out definition provides a broader definition.

"C_1-C_6 -alkyl" refers to monovalent alkyl groups having 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, *tert*-butyl, n-hexyl and the like.

"Aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (*e.g.*, phenyl) or multiple condensed rings (*e.g.*, naphthyl). Preferred aryl include phenyl, naphthyl, phenantrenyl and the like.

"C_1-C_6-alkyl aryl" refers to C_1-C_6-alkyl groups having an aryl substituent, including benzyl, phenethyl and the like.

"Heteroaryl" refers to a monocyclic heteroaromatic, or a bicyclic or a tricyclic fused-ring heteroaromatic group. Particular examples of heteroaromatic groups include optionally substituted pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl,1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, quinolizinyl, quinazolinyl, pthalazinyl, quinoxalinyl, cinnolinyl, napthyridinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5,6,7,8-tetrahydroisoquinolyl, purinyl, pteridinyl, carbazolyl, xanthenyl or benzoquinolyl.

"C_1-C_6-alkyl heteroaryl" refers to C_1-C_6-alkyl groups having a heteroaryl substituent, including 2-furylmethyl, 2-thienylmethyl, 2-(1H-indol-3-yl)ethyl and the like.

"C_2-C_6-alkenyl" refers to alkenyl groups preferably having from 2 to 6 carbon atoms and having at least 1 or 2 sites of alkenyl unsaturation. Preferable alkenyl groups include ethenyl ($-CH=CH_2$), n-2-propenyl (allyl, $-CH_2CH=CH_2$) and the like.

"C_2-C_6-alkenyl aryl" refers to C_2-C_6-alkenyl groups having an aryl substituent, including 2-phenylvinyl and the like.

"C_2-C_6-alkenyl heteroaryl" refers to C_2-C_6-alkenyl groups having a heteroaryl substituent, including 2-(3-pyridinyl)vinyl and the like.

"C_2-C_6-alkynyl" refers to alkynyl groups preferably having from 2 to 6 carbon atoms and having at least 1-2 sites of alkynyl unsaturation, preferred alkynyl groups include ethynyl (-C≡CH), propargyl (-CH_2C≡CH), and the like.

"C_2-C_6-alkynyl aryl" refers to C_2-C_6-alkynyl groups having an aryl substituent, including phenylethynyl and the like.

"C_2-C_6-alkynyl heteroaryl" refers to C_2-C_6-alkynyl groups having a heteroaryl substituent, including 2-thienylethynyl and the like.

"C_3-C_8-cycloalkyl" refers to a saturated carbocyclic group of from 3 to 8 carbon atoms having a single ring (*e.g.*, cyclohexyl) or multiple condensed rings (*e.g.*, norbornyl). Preferred cycloalkyl include cyclopentyl, cyclohexyl, norbornyl and the like.

"Heterocycloalkyl" refers to a C_3-C_8-cycloalkyl group according to the definition above, in which up to 3 carbon atoms are replaced by heteroatoms chosen from the group consisting of O, S, NR, R being defined as hydrogen or methyl. Preferred heterocycloalkyl include pyrrolidine, piperidine, piperazine, 1-methylpiperazine, morpholine, and the like.

"C_1-C_6-alkyl cycloalkyl" refers to C_1-C_6-alkyl groups having a cycloalkyl substituent, including cyclohexylmethyl, cyclopentylpropyl, and the like.

"C_1-C_6-alkyl heterocycloalkyl" refers to C_1-C_6-alkyl groups having a heterocycloalkyl substituent, including 2-(1-pyrrolidinyl)ethyl, 4-morpholinylmethyl, (1-methyl-4-piperidinyl)methyl and the like.

"Carboxy" refers to the group –C(O)OH.

"C_1-C_6-alkyl carboxy" refers to C_1-C_6-alkyl groups having an carboxy substituent, including 2-carboxyethyl and the like.

"Acyl" refers to the group –C(O)R where R includes "C_1-C_6-alkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl".

"C_1-C_6-alkyl acyl" refers to C_1-C_6-alkyl groups having an acyl substituent, including 2-acetylethyl and the like.

"Aryl acyl" refers to aryl groups having an acyl substituent, including 2-acetylphenyl and the like.

"Heteroaryl acyl" refers to hetereoaryl groups having an acyl substituent, including 2-acetylpyridyl and the like.

"C_3-C_8-(hetero)cycloalkyl acyl" refers to 3 to 8 memebered cycloalkyl or heterocycloalkyl groups having an acyl substituent.

"Acyloxy" refers to the group –OC(O)R where R includes H, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", heterocycloalkyl"heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl acyloxy" refers to C_1-C_6-alkyl groups having an acyloxy substituent, including 2-(acetyloxy)ethyl and the like.

"Alkoxy" refers to the group –O-R where R includes "C_1-C_6-alkyl" or "aryl" or "hetero-aryl" or "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl". Preferred alkoxy groups include by way of example, methoxy, ethoxy, phenoxy and the like.

"C_1-C_6-alkyl alkoxy" refers to C_1-C_6-alkyl groups having an alkoxy substituent, including 2-ethoxyethyl and the like.

"Alkoxycarbonyl" refers to the group –C(O)OR where R includes H, "C_1-C_6-alkyl" or "aryl" or "heteroaryl" or "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl".

"C_1-C_6-alkyl alkoxycarbonyl" refers to C_1-C_5-alkyl groups having an alkoxycarbonyl substituent, including 2-(benzyloxycarbonyl)ethyl and the like.

"Aminocarbonyl" refers to the group –C(O)NRR' where each R, R' includes independently hydrogen or C_1-C_6-alkyl or aryl or heteroaryl or "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl hetero-aryl".

"C_1-C_6-alkyl aminocarbonyl" refers to C_1-C_6-alkyl groups having an aminocarbonyl substituent, including 2-(dimethylaminocarbonyl)ethyl and the like.

"Acylamino" refers to the group –NRC(O)R' where each R, R' is independently hydrogen, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl acylamino" refers to C_1-C_6-alkyl groups having an acylamino substituent, including 2-(propionylamino)ethyl and the like.

"Ureido" refers to the group –NRC(O)NR'R" where each R, R', R" is independently hydrogen, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl", and where R' and R", together with the nitrogen atom to which they are attached, can optionally form a 3-8-membered heterocycloalkyl ring.

"C_1-C_6-alkyl ureido" refers to C_1-C_6-alkyl groups having an ureido substituent, including 2-(*N'*-methylureido)ethyl and the like.

"Carbamate" refers to the group –NRC(O)OR' where each R, R' is independently hydrogen, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"Amino" refers to the group –NRR' where each R,R' is independently hydrogen or "C_1-C_6-alkyl" or "aryl" or "heteroaryl" or "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", or "cycloalkyl", or "heterocycloalkyl", and where R and R', together with the nitrogen atom to which they are attached, can optionally form a 3-8-membered heterocycloalkyl ring.

"C_1-C_6-alkyl amino" refers to C_1-C_5-alkyl groups having an amino substituent, including 2-(1-pyrrolidinyl)ethyl and the like.

"Ammonium" refers to a positively charged group $-N^+RR'R''$, where each R,R',R'' is independently "C_1-C_6-alkyl" or "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", or "cycloalkyl", or "heterocycloalkyl", and where R and R', together with the nitrogen atom to which they are attached, can optionally form a 3-8-membered heterocycloalkyl ring.

"C_1-C_6-alkyl ammonium" refers to C_1-C_6-alkyl groups having an ammonium substituent, including 2-(1-pyrrolidinyl)ethyl and the like.

"Halogen" refers to fluoro, chloro, bromo and iodo atoms.

"Sulfonyloxy" refers to a group $-OSO_2$-R wherein R is selected from H, "C_1-C_6-alkyl", "C_1-C_6-alkyl" substituted with halogens, *e.g.*, an $-OSO_2$-CF_3 group, "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl sulfonyloxy" refers to C_1-C_5-alkyl groups having a sulfonyloxy substituent, including 2-(methylsulfonyloxy)ethyl and the like.

"Sulfonyl" refers to group "$-SO_2$-R" wherein R is selected from H, "aryl", "heteroaryl", "C_1-C_6-alkyl", "C_1-C_6-alkyl" substituted with halogens, *e.g.*, an $-SO_2$-CF_3 group, "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl

heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl sulfonyl" refers to C_1-C_5-alkyl groups having a sulfonyl substituent, including 2-(methylsulfonyl)ethyl and the like.

"Sulfinyl" refers to a group "–S(O)-R" wherein R is selected from H, "C_1-C_6-alkyl", "C_1-C_6-alkyl" substituted with halogens, *e.g.*, a –SO-CF_3 group, "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl sulfinyl" refers to C_1-C_5-alkyl groups having a sulfinyl substituent, including 2-(methylsulfinyl)ethyl and the like.

"Sulfanyl" refers to groups –S-R where R includes H, "C_1-C_6-alkyl", "C_1-C_6-alkyl" substituted with halogens, *e.g.*, a –SO-CF_3 group, "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl". Preferred sulfanyl groups include methylsulfanyl, ethylsulfanyl, and the like.

"C_1-C_6-alkyl sulfanyl" refers to C_1-C_5-alkyl groups having a sulfanyl substituent, including 2-(ethylsulfanyl)ethyl and the like.

"Sulfonylamino" refers to a group –$NRSO_2$-R' where each R, R' includes independently hydrogen, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl sulfonylamino" refers to C_1-C_5-alkyl groups having a sulfonylamino substituent, including 2-(ethylsulfonylamino)ethyl and the like.

"Aminosulfonyl" refers to a group $-SO_2$-NRR' where each R, R' includes independently hydrogen, "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "C_3-C_8-cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl", "C_1-C_6-alkyl aryl" or "C_1-C_6-alkyl heteroaryl", "C_2-C_6-alkenyl aryl", "C_2-C_6-alkenyl heteroaryl", "C_2-C_6-alkynyl aryl", "C_2-C_6-alkynylheteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl".

"C_1-C_6-alkyl aminosulfonyl" refers to C_1-C_6-alkyl groups having an aminosulfonyl substituent, including 2-(cyclohexylaminosulfonyl)ethyl and the like.

"Substituted or unsubstituted": Unless otherwise constrained by the definition of the individual substituent, the above set out groups, like "alkyl", "alkenyl", "alkynyl", "aryl" and "heteroaryl" etc. groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of "C_1-C_6-alkyl", "C_2-C_6-alkenyl", "C_2-C_6-alkynyl", "cycloalkyl", "heterocycloalkyl", "C_1-C_6-alkyl aryl", "C_1-C_6-alkyl heteroaryl", "C_1-C_6-alkyl cycloalkyl", "C_1-C_6-alkyl heterocycloalkyl", "amino", "ammonium", "acyl", "acyloxy", "acylamino", "aminocarbonyl", "alkoxycarbonyl", "ureido", "aryl", "carbamate", "heteroaryl", "sulfinyl", "sulfonyl", "alkoxy", "sulfanyl", "halogen", "carboxy", trihalomethyl, cyano, hydroxy, mercapto, nitro, and the like. Alternatively said substitution could also comprise situations where neighbouring substituents have undergone ring closure, notably when vicinal functional substituents are involved, thus forming, *e.g.*, lactams, lactons, cyclic anhydrides, but also acetals, thioacetals, aminals formed by ring closure for instance in an effort to obtain a protective group.

"Pharmaceutically acceptable cationic salts or complexes" is intended to define such salts as the alkali metal salts, (e.g. sodium and potassium), alkaline earth metal salts (e.g. calcium or magnesium), aluminium salts, ammonium salts and salts with organic amines such as with methylamine, dimethylamine, trimethylamine, ethylamine, triethylamine, morpholine, N-Me-D-glucamine, N,N'-bis(phenylmethyl)-1,2-ethanediamine,

ethanolamine, diethanolamine, ethylenediamine, N-methylmorpholine, piperidine, benzathine (N,N'-dibenzylethylenediamine), choline, ethylene-diamine, meglumine (N-methylglucamine), benethamine (N-benzylphenethylamine), diethylamine, piperazine, thromethamine (2-amino-2-hydroxymethyl-1,3-propanediol), procaine as well as amines of formula –NR,R',R'' wherein R, R', R'' is independently hydrogen, alkyl or benzyl. Especially preferred salts are sodium and potassium salts.

"Pharmaceutically acceptable salts or complexes" refers to salts or complexes of the below-identified compounds of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) that retain the desired biological activity. Examples of such salts include, but are not restricted to acid addition salts formed with inorganic acids (*e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, fumaric acid, maleic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene sulfonic acid, naphthalene disulfonic acid, and poly-galacturonic acid. Said compounds can also be administered as pharmaceutically acceptable quaternary salts known by a person skilled in the art, which specifically include the quarternary ammonium salt of the formula –NR,R',R'' $^{+}$ Z^{-}, wherein R, R', R'' is independently hydrogen, alkyl, or benzyl, C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_1-C_6-alkyl aryl, C_1-C_6-alkyl heteroaryl, cycloalkyl, heterocycloalkyl, and Z is a counterion, including chloride, bromide, iodide, -O-alkyl, toluenesulfonate, methylsulfonate, sulfonate, phosphate, or carboxylate (such as benzoate, succinate, acetate, glycolate, maleate, malate, fumarate, citrate, tartrate, ascorbate, cinnamoate, mandeloate, and diphenylacetate).

"Pharmaceutically active derivative" refers to any compound that upon administration to the recipient, is capable of providing directly or indirectly, the activity disclosed herein.

"Enantiomeric excess" (ee) refers to the products that are obtained by an asymmetric synthesis, i.e. a synthesis involving non-racemic starting materials and/or reagents or a synthesis comprising at least one enantioselective step, whereby a surplus of one enantiomer in the order of at least about 52% ee is yielded.

"Spermatozoa" or "sperm (cells)" are used synonymously herein and relate to male gametes. "Semen" or "seminal fluid/liquid" contain sperm cells as well as seminal plasma.

"Increase of spermatozoa fertilization activity" refers to any enhancement, improvement, or change to the better of the parameters determining the quality or activity of the sperm cell, such as e.g. percentage curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL) and hyperactivated sperm fraction (HA). The quality of the spermatozoa determines the fertilization rate in assisted reproduction techniques.

"Increase of spermatozoa motility" refers to any improvement, enhancement, amelioration or change to the better of the quality or fertilization activity or motility or velocity of the cells.

"Phosphatidylinositol-3-kinase" or "PI3K" refers to any member of the PI3K family, i.e. those related enzymes having the activity outlined in the indroduction.

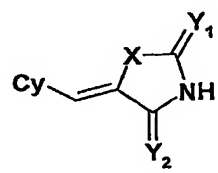
"Inhibitor of phosphatidylinositol-3-kinase" refers to as PI3K and inhibits the production of D-3 phosphoinositides in the cell. The term D-3 phosphoinositides is intended to encompass derivatives of phosphatidylinositol that are phosphorylated in the D-3 position of the inositol ring and comprises, for example, phosphatidylinositol(3)monophosphate (PI(3)P), phosphatidylinositol(3,4)bisphosphate ($PI(3,4)P_2$) or phosphatidylinositol-(3,4,5)trisphosphate ($PI(3,4,5)P_3$).

"Effective amount" refers to an amount of the active ingredients that is sufficient to affect the fertilization activity, in particular the mobility of spermatozoa. The effective amount will depend on the route of administration and the condition of the patient.

"Pharmaceutically acceptable" refers to any carrier, which does not interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered. For example, for parenteral administration, the above active ingredients may be formulated in unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution. Besides the pharmaceutically acceptable carrier, the compositions of the invention can also

comprise minor amounts of common additives, such as stabilisers, excipients, buffers and preservatives.

According to the present invention, said process to improve the spermatozoa fertilization activity, in particular for increasing spermatozoa motility, comprises the step of treating spermatozoa with a compound of formula (I).



(I)

Formula (I) also comprises its geometrical isomers, its optically active forms as enantiomers, diastereomers and its racemate forms, as well as pharmaceutically acceptable salts and pharmaceutically active derivatives thereof. Preferred pharmaceutically acceptable salts of the formula (I) are acid addition salts formed with pharmaceutically acceptable acids, like hydrochloride, hydrobromide, sulfate or bisulfate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methanesulfonate, benzenesulfonate, and *para*-toluenesulfonate salts.

Such compounds of formula (I) may be used for the preparation of a pharmaceutical composition to improve the spermatozoa fertilization activity, in particular to increase spermatozoa motility and for the treatment of spermatozoa.

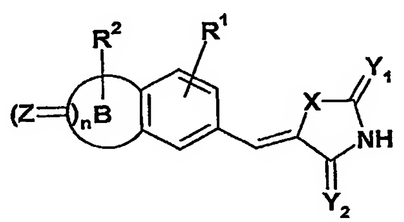
The substituents within formula (I) are defined as follows :

X is S, O or NH, preferably S.

Y^1 and Y^2 are independently S, O or -NH, preferably O.

Cy is a substituted or unsubstituted 5 to 8 membered carbocyclic or heterocyclic group which may be optionally fused with an aryl, heteroaryl, cycloalkyl or heterocycloalkyl ring.

According to a more specific embodiment of the invention, the compounds of formula (I) have a fused phenyl moiety thus giving compounds of formula (I').



(I')

B is a 5-8 membered heterocyclic ring or a carbocyclic group, wherein said carbocyclic group may be fused with an aryl, heteroaryl, cycloalkyl or heterocycloalkyl group and X, Y^1, Y^2 are as above-defined.

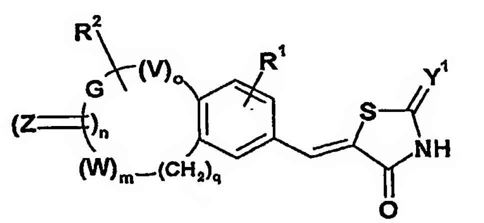
Z is O or S, preferably O and n is 0, 1 or 2.

R^1 is H, CN, carboxy, acyl, C_1-C_6-alkoxy, halogen, hydroxy, acyloxy, an unsubstituted or substituted C_1-C_6-alkyl carboxy, an unsubstituted or substituted C_1-C_6-alkyl acyloxy, an unsubstituted or substituted C_1-C_6-alkyl alkoxy, alkoxycarbonyl, an unsubstituted or substituted C_1-C_6-alkyl alkoxycarbonyl, aminocarbonyl, an unsubstituted or substituted C_1-C_6-alkyl aminocarbonyl, acylamino, an unsubstituted or substituted C_1-C_6-alkyl acylamino, ureido, an unsubstituted or substituted C_1-C_6-alkyl ureido, amino, an unsubstituted or substituted C_1-C_6-alkyl amino, ammonium, sulfonyloxy, an unsubstituted or substituted C_1-C_6-alkyl sulfonyloxy, sulfonyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonyl, sulfinyl, an unsubstituted or substituted C_1-C_6-alkyl sulfinyl, sulfanyl, an unsubstituted or

substituted C_1-C_6-alkyl sulfanyl, sulfonylamino, an unsubstituted or substituted C_1-C_6-alkyl sulfonylamino or carbamate. Preferably R^1 is H.

R^2 is selected from the group comprising or consisting of H, halogen, acyl, amino, an unsubstituted or substituted C_1-C_6-alkyl, an unsubstituted or substituted C_2-C_6-alkenyl, an unsubstituted or substituted C_2-C_6-alkynyl, an unsubstituted or substituted C_1-C_6-alkyl carboxy, an unsubstituted or substituted C_1-C_6-alkyl acyl, an unsubstituted or substituted C_1-C_6-alkyl alkoxycarbonyl, an unsubstituted or substituted C_1-C_6-alkyl aminocarbonyl, an unsubstituted or substituted C_1-C_6-alkyl acyloxy, an unsubstituted or substituted C_1-C_6-alkyl acylamino, an unsubstituted or substituted C_1-C_6-alkyl ureido, an unsubstituted or substituted C_1-C_6-alkyl amino, an unsubstituted or substituted C_1-C_6-alkyl alkoxy, an unsubstituted or substituted C_1-C_6-alkyl sulfanyl, an unsubstituted or substituted C_1-C_6-alkyl sulfinyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonylaminoaryl, an unsubstituted or substituted aryl, an unsubstituted or substituted heteroaryl, an unsubstituted or substituted C_3-C_8-cycloalkyl or an unsubstituted or substituted heterocycloalkyl, an unsubstituted or substituted C_1-C_6-alkyl aryl, an unsubstituted or substituted C_1-C_6-alkyl heteroaryl, an unsubstituted or substituted C_2-C_6-alkenyl-aryl or -heteroaryl, an unsubstituted or substituted C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, cyano, hydroxy, C_1-C_6-alkoxy, nitro, acylamino, ureido, sulfonylamino, sulfanyl, or sulfonyl.

A further particularly preferred aspect of the present invention is related to the use of thiazolidinedione-vinyl fused-benzene derivatives of any of formulae (Ia), (Ib), (Ic) and (Id):



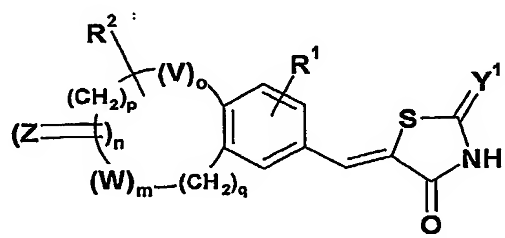
(Ia)

Y^1, Z; R^1, R^2 and n in formula (Ia) are as above-defined.

G in formula (Ia) is an unsubstituted or substituted C_1-C_5 alkylene (e.g. methylene, ethylene, propylene etc.) or an unsubstituted or substituted C_1-C_5 alkenylene group (e.g. a methine (-CH=), a -CH=CH- group, a propenylene group, etc.).

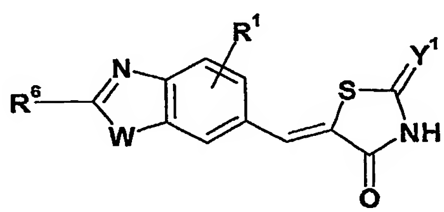
W and V in formula (Ia) are each independently from each other selected from O, S, $-NR^3$ wherein R^3 is H or an unsubstituted or substituted C_1-C_6 alkyl group, m and o are each independently from each other 0 or 1, p is an integer from 1 to 4 and q is an integer from 0 to 4.

Even more preferred compounds of formula (Ia) is where G is a C_1-C_4 alkylene, thus giving compounds of formula (Ib) (i.e. p = 1, 2, 3 or 4, preferably 1 or 2).



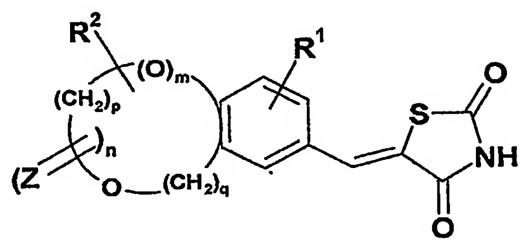
(Ib)

A particularly preferred sub-group of formula (Ib) are compounds having the formula (Ic), whereby W is as above defined and R^6 is H or OH.



(Ic)

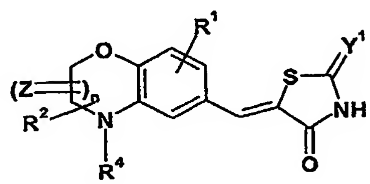
Still a further preferred sub-group of formula (Ia) are compounds, wherein V, W and Y^1 are all O, thus providing compounds of formula (Id).



(Id)

In a preferred embodiment of formulae (Ia), (Ib) or (Id), m is 0, n is 1, p is 1 or 2, q is 1, Z is O and R^1, R^2 is as above-defined.

Other preferred thiazolidinedione-vinyl benzene-fused derivatives for the above-mentioned process according to the invention are those of formula (II)



(II)

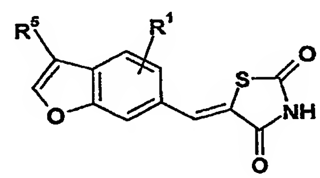
as well as its geometrical isomers, its optically active forms as enantiomers, diastereomers and its racemate forms, as well as pharmaceutically acceptable forms, as well as pharmaceutically active derivatives thereof,

wherein Z, Y^1, R^1, R^2 are as above defined. n is 0 or 1.

R^4 is selected in the group comprising or consisting of H, acyl, an unsubstituted or substituted C_1-C_6-alkyl, an unsubstituted or substituted C_2-C_6-alkenyl, an unsubstituted or substituted C_2-C_6-alkynyl, an unsubstituted or. substituted C_1-C_6-alkyl carboxy, an unsubstituted or substituted C_1-C_6-alkyl acyl, an unsubstituted or substituted C_1-C_6-alkyl alkoxycarbonyl, an unsubstituted or substituted C_1-C_6-alkyl aminocarbonyl, an unsubstituted or substituted C_1-C_6-alkyl acyloxy, an unsubstituted or substituted C_1-C_6-

alkyl, acylamino, an unsubstituted or substituted C_1-C_6-alkyl ureido, an unsubstituted or substituted C_1-C_6-alkyl amino, an unsubstituted or substituted C_1-C_6-alkyl alkoxy or an unsubstituted or substituted C_1-C_6-alkyl sulfanyl, an unsubstituted or substituted C_1-C_6-alkyl sulfinyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonylaminoaryl, an unsubstituted or substituted aryl, an unsubstituted or substituted heteroaryl, an unsubstituted or substituted C_3-C_8-cycloalkyl or heterocycloalkyl, an unsubstituted or substituted C_1-C_6-alkyl aryl, an unsubstituted or substituted C_1-C_6-alkyl heteroaryl, an unsubstituted or substituted C_2-C_6-alkenyl-aryl or -heteroaryl, an unsubstituted or substituted C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, hydroxy, C_1-C_6-alkoxy, C_1-C_6 alkyl carbamate, sulfonylamino, sulfanyl or sulfonyl.

Further preferred thiazolidinedione-vinyl benzene-fused derivatives for the above-mentioned process according to the invention are those of formula (III)



(III)

as well as its geometrical isomers, its optically active forms as enantiomers, diastereomers and its racemate forms, as well as pharmaceutically acceptable forms, as well as pharmaceutically active derivatives thereof.

R^1 is as above defined and R^5 is selected in the group comprising or consisting of H, halogen, acyl, amino, an unsubstituted or substituted C_1-C_6-alkyl, an unsubstituted or substituted C_2-C_6-alkenyl, an unsubstituted or substituted C_2-C_6-alkynyl, an unsubstituted or substituted C_1-C_6-alkyl carboxy, an unsubstituted or substituted C_1-C_6-alkyl acyl, an unsubstituted or substituted C_1-C_6-alkyl alkoxycarbonyl, an unsubstituted or substituted C_1-C_6-alkyl aminocarbonyl, an unsubstituted or substituted C_1-C_6-alkyl acyloxy, an unsubstituted or substituted C_1-C_6-alkyl, acylamino, an unsubstituted or substituted C_1-C_6-alkyl ureido, an unsubstituted or substituted C_1-C_6-alkyl amino, an unsubstituted or substituted C_1-C_6-alkyl alkoxy or an unsubstituted or substituted C_1-C_6-alkyl sulfanyl, an

unsubstituted or substituted C_1-C_6-alkyl sulfinyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonyl, an unsubstituted or substituted C_1-C_6-alkyl sulfonylaminoaryl, an unsubstituted or substituted aryl, an unsubstituted or substituted heteroaryl, an unsubstituted or substituted C_3-C_8-cycloalkyl or heterocycloalkyl, an unsubstituted or substituted C_1-C_6-alkyl aryl, an unsubstituted or substituted C_1-C_6-alkyl heteroaryl, an unsubstituted or substituted C_2-C_6-alkenyl-aryl or -heteroaryl, an unsubstituted or substituted C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, cyano, hydroxy, C_1-C_6-alkoxy, nitro, acylamino, C_1-C_6 alkyl carbamate, ureido, sulfonylamino, sulfanyl or sulfonyl.

A preferred aspect according to the invention is the one wherein the compounds of formula (I) are selected from the group consisting of:

(5E)-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one;

(5Z)-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-1,3-thiazolidine-2,4-dione;

(5Z)-5-(2,3-dihydro-1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione;

(5E)-5-[(7-methoxy-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione;

(5Z)-5-[(9,10-dioxo-9,10-dihydroanthracen-2-yl)methylene]-1,3-thiazolidine-2,4-dione;

(5Z)-5-[(2,2-difluoro-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione;

(5Z)-5-(1,3-dihydro-2-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione;

(5Z)-5-(1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione;

(5Z)-5-[(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)methylene]-1,3-thiazolidine-2,4-dione;

(5Z)-5-[(4-methyl-3,4-dihydro-2H-1,4-benzoxazin-7-yl)methylene]-1,3-thiazolidine-2,4-dione;

(5Z)-5-(1,3-benzodioxol-5-ylmethylene)-2-imino-1,3 -thiazolidin-4-one..

These agents have been shown to be particularly efficacious for the enhancement of sperm fertilization activity.

Preferably, the spermatozoa are treated with an amount of a compound of any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) in the range of about 0.01 to 1000 µM, more preferably of about 5 to 500 µM and most preferably of about 10 to 100 µM. Treating the spermatozoa with a compound of formula (I) advantageously comprises incubating the spermatozoa for a period of time in the range of about 30 minutes to 10 hours, preferably about 1 to 8 hours, most preferably about 2 to 6 hours at a temperature of about 37°C.

The invention is based on the finding that phosphatidylinositol-3-kinase inhibitors have a pronounced positive effect on parameters determining sperm cell fertilization activity, i.e. the parameters relevant to the capacity of sperm cells to fertilize an oozyte. The most important factors involved in the ability to fertilize are the number of active sperms and the motility of the spermatozoa. According to the WHO manual, motility of 50% is considered the lower limit of normality.

It has now been found in accordance with the invention that the number of motile sperms obtainable from semen samples as well as the motility of the individual spermatozoa can be significantly increased by using compounds of formula (I). This effect is detectable in normospermic individuals. However, it is even more marked in spermatozoa displaying pathogenic features, like oligoasthenospermic patients, i.e. those patients having a reduced total number of spermatozoa and a reduced spermatozoa motility. The invention renders it possible to increase the percentage of spermatozoa with progressive motility, thus significantly improving the probability of successful fertilization. Thus, the process according to the invention helps patients avoid using ICSI in favor of less invasive ART, like conventional IVF.

In a preferred embodiment, treating the spermatozoa with a compound of formula (I) is performed on the seminal liquid comprising the spermatozoa. Performing the method according to the invention directly on the seminal liquid without any further treatment has

the advantage that it is simple and fast. Since the PI3K inhibitor of the invention enhances sperm cell motility, removal of the seminal plasma is not necessary.

In a further preferred embodiment, the process further comprises separating the spermatozoa by spermatozoa separation methods used in assisted reproduction techniques (ART).

Since seminal plasma contains factors that inhibit capacitation and fertilization as well as a considerable amount of non-motile spermatozoa even in a fertile individual, it is advantageous to separate motile sperm cells from fluid, non-motile and morphologically defective spermatozoa. This step is essential in traditional ART like IVF, GIFT or Intra-uterine Insemination (IUI). It leads to an enhancement of the fertilization success rate also in the process according to the invention. It is evident from the examples that the increase in spermatozoa motility by using a compound of formula (I) is even more pronounced in spermatozoa which have been separated from the seminal plasma.

In a further preferred embodiment of the invention, separating the spermatozoa is performed by a method selected from the wash and spin method, the sedimentation method, the direct swim-up method, the pellet and swim-up method, and the buoyant density gradient method. These methods are well known in the art. They are traditionally used in assisted reproduction techniques and described in detail in "A textbook of In Vitro Fertilization and Assisted Reproduction, The Bourn Hall guide to clinical and laboratory practice, editor: Peter R. Brinsden, The Parthenon Publishing Group" (1999) on pages 204 to 208. This textbook is referred to hereinafter as the "Bourn Hall guide".

Preferably, separating the spermatozoa is performed by the direct swim-up method. This method implies self-selection of motile sperms, essentially comprising layering an aliquot of medium on top of a semen sample and allowing it to stand a room temperature for a certain period of time. The motile sperm cells will migrate into the top layer (medium), from which they can be recovered. The method may also include centrifugation step(s). The advantage of "swim-up" selected spermatozoa is that the motile cells present in the sample are isolated and concentrated and that the proportion of morphologically normal

sperm is increased. It is shown in the examples that the process according to the invention leads to an increase of the amount of spermatozoa recovered from seminal fluid by the swim-up method. This is due to the increased motility of the sperms, which therefore migrate more quickly and in higher amounts into the upper phase of the sample.

The method may be varied and combined with further isolation/separation techniques, depending on the amount of motile cells in the sample. For example, the swim-up procedure may be performed through the layering of 1 ml of medium containing albumin on a 1 ml of underlying seminal liquid in a test tube. After one hour of incubation at 37°C in the air or in 5% CO_2 the upper phase of the medium to which the spermatozoa with better motility characteristics have migrated is collected. This technique may also comprise or be combined with a centrifugation step, for example centrifugation on Percoll gradients. The separated, isolated or enriched spermatozoa are then used in assisted-reproduction techniques or may be deep-frozen before being further processed, for example.

Advantageously, the incubation of spermatozoa with a compound of formula (I) is carried out on the seminal fluid, and then swim-up selection is performed. Thereafter, the spermatozoa may be washed one or several times to eliminate the compound of formula (I), before being further processed for fertilization.

Preferably, the process according to the invention is performed on mammal spermatozoa, in particular on human spermatozoa.

The invention also relates to spermatozoa obtainable by the process described above. It is a further object of the invention to provide spermatozoa having an improved ability of fertilization. Therefore the invention further relates to spermatozoa in which the activity of the phosphatidylinositol-3 kinase is inhibited. The spermatozoa in which the a compound of formula (I) is inhibited or which were obtained in a process according to the invention exhibit an improved fertilization activity, a higher motility as compared to untreated sperm cells and thus exhibit a better performance with regard to fertilization.

As above-mentioned, sperm cell fertilization activity determines the fertilization rate in ART. The invention therefore further relates to the use of a compound of the above-mentioned formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) for improving the fertilization rate in assisted reproduction techniques.

Any assisted reproduction method known in the art may be used according to the invention. In preferred embodiments, the assisted reproduction techniques are selected from in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), and intra-uterine insemination (IUI).

The invention further relates to the use of a compound of formula (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) for the preparation of a pharmaceutical composition for the treatment of infertility, in particular male infertility. While the invention is described in more detail for in vitro fertilization techniques, it will be appreciated by the person skilled in the art that the compound may be as efficient in terms of activity when administered *in vivo*.

In this case, the medicament is preferably presented in the form of a pharmaceutical composition comprising a compound of formula (I) together with one or more pharmaceutically acceptable carriers and/or excipients. Such pharmaceutical compositions form yet a further aspect of the present invention.

The administration of such active ingredient may be by intravenous, intramuscular or subcutaneous route. Other routes of administration, which may establish the desired blood levels of the respective ingredients, are comprised by the present invention.

The invention further relates to the use of a compound of formula (I), (I'), (Ia), (Ib), (Ic), (Id), (II) and (III) for the preparation of a pharmaceutical composition for the improvement of spermatozoa fertilization activity, in particular for the increase of spermatozoa motility.

It is a further object of the present invention to provide for an improvement concerning the method of ART therapy. The improvement consists in including into known techniques for assisted fertilization a step comprising treating spermatozoa with a compound of formula (I), (I'), (Ia), (Ib), (Ic), (Id), (II) and (III). The further steps used in assisted reproduction

techniques are well known to the person skilled in the art and can be taken form the WHO manual (supra) or the Bourn Hall guide (supra).

In a preferred embodiment of the invention, the ART are selected from in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), or intra-uterine insemination (IUI).

It is a further object of the present invention to provide a medium for storage and/or transportation of mammal spermatozoa, particular human spermatozoa, having improved qualities. The invention therefore also relates to a medium comprising a compound of formula (I), (I'), (Ia), (Ib), (Ic), (Id), (II) and (III). Apart from the a compound of formula (I), the medium may contain any further component known to be useful for storage and/or transportation, depending on the kind of storage and/or transportation required. For example, the spermatozoa may be stored at room temperature or by cryo-preservation. The latter is common for the storage of the cells for a longer period of time. Specific examples of further components of the medium can be taken e.g. from WO 97/16965. Further specific media suitable for cryopreservation of semen are included in Appendix II, pp. 541 and 542 of the Bourn Hall guide (supra), for instance. They could be supplemented with a compound of formula (I) to improve the fertilization activity, in particular the motility of the sperm before fertilization takes place.

In a preferred embodiment, the medium comprises mammal spermatozoa, in particular human spermatozoa. Preferable, a compound of formula (I) present in the medium according to the invention is selected from the group consisting of (5-(2H-benzo[d]1,3-dioxolen-5-ylmethylene)-1,3-thiazolidine-2,4-dione and derivatives and analogues thereof. In a highly preferred embodiment, the compound of formula (I) is (5-(2H-benzo[d]1,3-dioxolen-5-ylmethylene)-1,3-thiazolidine-2,4-dione.

In yet a further preferred embodiment, the medium according to the invention comprises amounts of the compound of formula (I), (I'), (Ia), (Ib), (Ic), (Id), (II) and (III) in the range of about 0.01 to 1000 μM, preferably of about 5 to 500 μM, and most preferably of about 10 to 100 μM.

Having now described the invention, it will be more readily understood through reference to the following examples that are provided by way of illustration and are not intended to be limiting the present invention.

Compounds of formula (I), in particular those of formulae (I'), (Ia), (Ib), (Ic), (Id), (II) and (III), have been found - in accordance with the present invention - to be PI3K inhibitors.

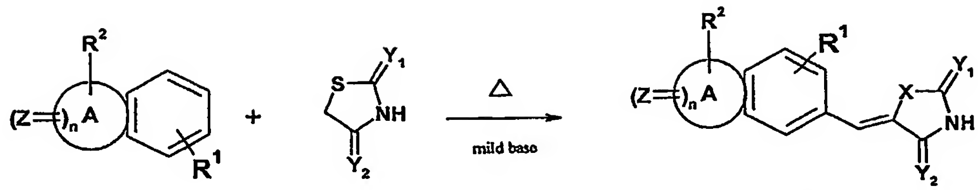
The azolidinone-vinyl fused-benzene derivatives according to formula (I) are either commercially available or - as is the case for compounds of any of formulae (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) - may be prepared from readily available starting materials using the below set out general methods and procedures.

It will be appreciated that where typical or preferred experimental conditions (i.e. reaction temperatures, time, moles of reagents, solvents etc.) are given, other experimental conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvents used, but such conditions can be determined by the person skilled in the art, using routine optimisation procedures.

In the process illustrated in the following schemes R^1, R^2, R^4, R^5, G, V, W, Y^1, Y^2, Z, m, n, o, p and q are each as above-defined in the description.

Generally, the azolidinone-vinyl fused-benzene derivatives according to the general formula (I') could be obtained by several synthetic approaches, using both solution-phase and solid-phase chemistry protocols (Brummond et.al., *J.O.C.*, **64**, 1723-1726 (1999)), either by conventional methods or by microwave-assisted techniques.

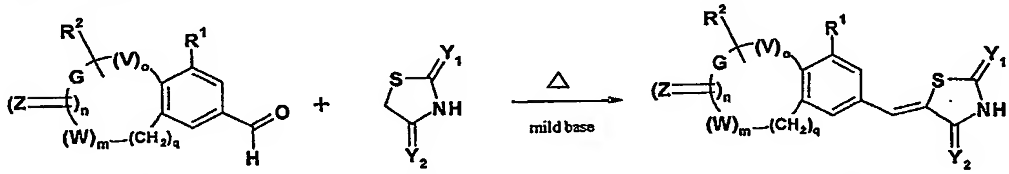
In a first step, approximately equimolar amounts of the reactant P1 and thiazolidinedione or rhodanin P are heated in the presence of a mild base to provide the corresponding olefin of formula (I').



P1 P (I')

Scheme 1

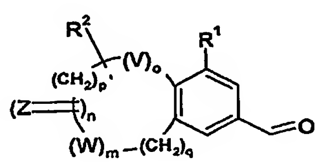
A preferred process according to the invention is illustrated by the following scheme 2 in which compounds of formula (Ia) respectively, may be obtained using the same reaction as above-mentioned.



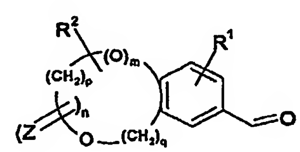
P1a P3 (Ia)

Scheme 2

P1a may be replaced with the following P1b and P1c in order to obtain the Formulae (Ib) and (Ic) respectively as above mentioned.

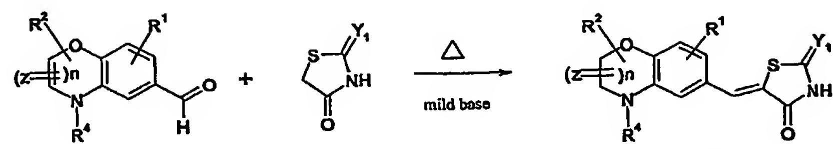


P1b



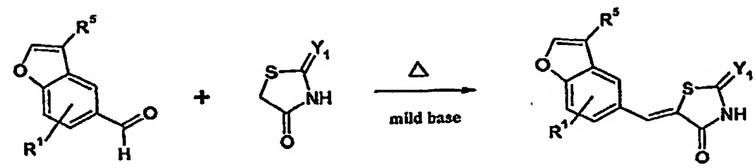
P1c

A particularly preferred process according to the invention are illustrated by the following schemes 3 and 4 in which compounds of formulae (II) and (III) respectively, may be obtained using the same reaction as above-mentioned.



P2 P3' (II)

Scheme 3



(III)

Scheme 4

While this step may be carried out in the absence of a solvent at a temperature, which is sufficiently high to cause at least partial melting of the reaction mixture, it is preferably carried out in the presence of a reaction inert solvent. A preferred such temperature is in the range of from 100°C to 250°C, and especially preferred is a temperature of from 120°C to 200°C. Examples of such solvents for the above reaction include solvents like dimethoxymethane, xylene, toluene, o-dichlorobenzene etc. Examples of suitable mild bases for the above reaction are alkali metal and alkaline earth salts of week acids such as the (C_1-C_{12})-alkyl carboxylic acids and benzoic acid, alkali metal and alkaline earth carbonates and bicarbonates such as calcium carbonate, magnesium carbonate, potassium bicarbonate and secondary amines such as piperidine, morpholine as well as tertiary amines such as pyridine, triethylamine, diisopropylethylamine, N-methylmorpholine, N-ethylpiperidine, N-methylpiperidine and the like. Especially preferred mild bases are sodium acetate or piperidine for reasons of economy and efficiency.

In a typical such reaction (Tietze et.al., in "The Knoevenagel Reaction", p.341 ff., Pergamon Press, Oxford 1991, Eds.: Trost B.M., Fleming I.) the aldehyde starting material P1a and thiazolidinedione P3 are combined in approximately equimolar amounts with 0.5

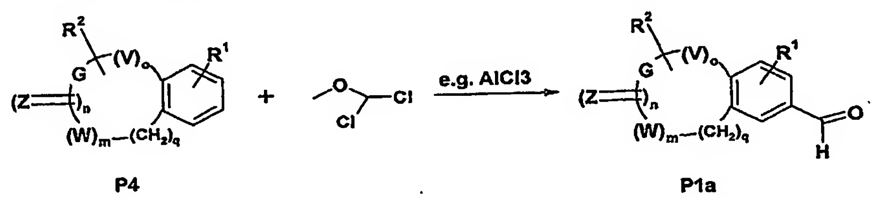
to one equivalent of piperidine in dimethoxymethane or similar solvent and heated between 120 and 200°C at which the reaction is substantially complete in from 15 minutes to 3 hours. The desired olefin of formula (Ia) is then isolated by filtration, in case it precipitated out of the reaction mixture upon cooling, or for example, by mixing with water and subsequent filtration, to obtain the crude product, which is purified, if desired, e.g. by crystallization or by standard chromatographic methods.

Alternatively olefins of formula (Ia) may be obtained typically by mixing equimolar amounts of thiazolidinedione P3 with aldheyde P1a and molar excess, preferably a 2-4 fold excess, of anhydrous sodium acetate and the mixture is heated at a temperature high enough to effect melting, at which temperature the reaction is mainly complete in from 5 to 60 minutes. Alternatively the above reaction can be carried out in acidic media such as acetic acid in the presence of sodium acetate.

Above described reaction can be carried out alternatively under microwave conditions as heating source. Typically the aldehyde starting material P1a and thiazolidinedione P3 are combined in approximately equimolar amounts with 0.5 to one equivalent of piperidine in dimethoxymethane or similar solvent and heated between 140°C and 240°C at which the reaction is substantially complete in from 3 to 10 minutes.

The pharmaceutically acceptable cationic salts of compounds of the present invention are readily prepared by reacting the acid forms with an appropriate base, usually one equivalent, in a co-solvent. Typical bases are sodium hxdroxide, sodium methoxide, sodium ethoxide, sodium hydride, potassium hydroxide, potassium methoxide, magnesium hydroxide, calcium hydroxide, benzathine, choline, diethanolamine, ethylenediamine, meglumine, benethamine, diethylamine, piperazine and tromethamine. The salt is isolated by concentration to dryness or by addition of a non-solvent. In some cases, salts can be prepared by mixing a solution of the acid with a solution of the cation (sodium ethylhexanoate, magnesium oleate), employing a solvent in which the desired cationic salt precipitates, or can be otherwise isolated by concentration and addition of a non-solvent.

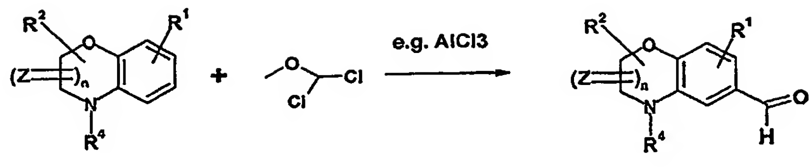
2,4-Thiazolidinedione P3 is commercially available from various sources. The aldehydes of formula P1a are prepared by a variety of well known methods, for example starting from the corresponding carboxylic acid alkyl ester or carboxylic acid by oxido-reduction, using standard techniques to reduce carboxylic acid alkyl ester or carboxylic acid to benzylic alcohols with Lihium aluminium hydride, Diisopropylaluminum etc. and ultimately re-oxidize the corresponding benzylic alcohol to the corresponding aldehyde by mild oxidation with reagents such as manganese dioxide, chromic acid, Dess-Martin reagent or Swern oxidation, or under conditions known to produce aldehydes from primary alcohols. An alternative way may be the direct reduction of the corresponding carboxylic acid alkyl ester or carboxylic acid to the corresponding aldehyde, using DIBAL at low temperature or any other techniques known in the field.



Scheme 5

An alternative way to produce the appropriate aldehydes is the selective reduction of a nitrile moiety to the corresponding aldehyde using known methods like e.g. DIBAL etc. Another alternative way to produce the appropriate aldehydes is the reaction of the corresponding benzene derivative in a Friedl-Crafts type of reaction wherein the substrate P4 as shown in the above scheme 5 is reacted with 1,1-dichloromethylmethyl ether in the presence of a Lewis acid such as titanium tetrachloride or aluminium trichloride or any corresponding Lewis acids suitable for such type of reaction.

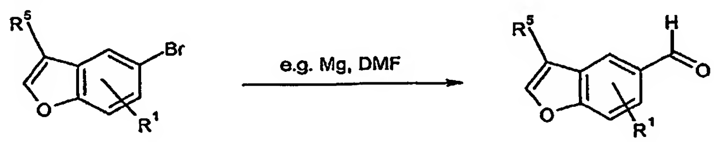
Acccording to a more particularly preferred process of the invention, as described in the literature (Petrov O.I., Kalcheva V.B., Antonova A.T., *Collect. Czech. Chem. Commun*, 62, p.494-7 (1997)) and illustrated by Scheme 6 hereinafter, reactant P2 may be obtained starting from P5 by reacting with 1,1-dichloromethylmethyl ether as above-described.



P5 P2

Scheme 6

According to another more particularly preferred process of the invention, as illustrated by Scheme 7 hereinafter, reactant P6 may be obtained starting from P7 by reacting with DMF and the presence of magnesium or *n*-butyl-lithium or any other method known to the person skilled in the art.



P_7 P_6

Scheme 7

If the above set out general synthetic methods are not applicable to obtain compounds according to formula (I) and/or to necessary intermediates for the synthesis of compounds of formula (I), suitable methods of preparation known by a person skilled on the art should be used. In general, the synthesis pathways for any individual compound of formula (I) will depend on the specific substitutents of each molecule and upon the ready availability of intermediates necessary; again such factors being appreciated by those of ordinary skill in the art. For all the protection and deprotection methods, see Philip J. Kocienski, in "*Protecting Groups*", Georg Thieme Verlag Stuttgart, New York, 1994 and, Theodora W. Greene and Peter G. M. Wuts in "*Protective Groups in Organic Synthesis*", Wiley Interscience, 3rd Edition 1993.

Compounds of this invention can be isolated in association with solvent molecules by crystallization from evaporation of an appropriate solvent. The pharmaceutically acceptable

acid addition salts of the compounds of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) which contain a basic center, may be prepared in a conventional manner. For example, a solution of the free base may be treated with a suitable acid, either neat or in a suitable solution, and the resulting salt isolated either by filtration or by evaporation under vacuum of the reaction solvent. Pharmaceutically acceptable base addition salts may be obtained in an analogous manner by treating a solution of compound of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) with a suitable base. Both types of salts may be formed or interconverted using ion-exchange resin techniques.

When employed as pharmaceuticals, azolidinedione-vinyl fused-benzene derivatives of the present invention are typically administered in the form of a pharmaceutical composition. Hence, pharmaceutical compositions comprising a compound of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) and a pharmaceutically acceptable carrier, diluent or excipient therefore are also within the scope of the present invention. A person skilled in the art is aware of a whole variety of such carrier, diluent or excipient compounds suitable to formulate a pharmaceutical composition.

The compounds of the invention, together with a conventionally employed adjuvant, carrier, diluent or excipient may be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, or capsules filled with the same, all for oral use, or in the form of sterile injectable solutions for parenteral (including subcutaneous use). Such pharmaceutical compositions and unit dosage forms thereof may comprise ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

Pharmaceutical compositions containing azolidinedione-vinyl fused-benzene derivatives of this invention can be prepared in a manner well known in the pharmaceutical art and comprise at least one active compound. Generally, the compounds of this invention are

administered in a pharmaceutically effective amount. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

The pharmaceutical compositions of the present invention can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular and intranasal. The compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include prefilled, premeasured ampoules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the thiazolidinedione-vinyl fused-benzene derivative is usually a minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

Liquid forms suitable for oral administration may include a suitable aqueous or nonaqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatine; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dio-xide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as pepper-mint, methyl salicylate, or orange flavoring.

Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. As above mentioned, the thiazolidinedione-vinyl fused-benzene derivatives of formula (I) in such compositions is typically a minor component, frequently ranging between 0.05 to 10% by weight with the remainder being the injectable carrier and the like.

The above described components for orally administered or injectable compositions are merely representative. Further materials as well as processing techniques and the like are set out in Part 5 of *Remington's Pharmaceutical Sciences*, 20th Edition, 2000, Marck Publishing Company, Easton, Pennsylvania, which is incorporated herein by reference. The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials can also be found in the incorporated materials in *Remington's Pharmaceutical Sciences*.

In the following the present invention shall be illustrated by means of some examples which are not construed to be viewed as limiting the scope of the invention. The following abbreviations are hereinafter used in the accompanying examples: min (minute), hr (hour), g (gram), mmol (millimole), m.p. (melting point), eq (equivalents), ml (milliliter), µl (microliters), ACN (acetonitrile), Boc (butoxycarbonyl), Cbz (carboxybenzyl), $CDCl_3$ (deuterated chloroform), cHex (cyclohexanes), dba (dibenzylidene acetone), DCM (dichloromethane), DEAD (diethylazodicarboxylate, DIC (diisopropyl carbodiimide), DIEA (diisopropyl ethylamine), DMAP (4-dimethylaminopyridine), DME (Dimethoxyethane), DMF (dimethylformamide), DMSO (dimethylsulfoxide), DMSO-d_6 (deuterated dimethylsulfoxide), EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), EtOAc (ethyl acetate), Et_2O (diethyl ether), Fmoc (9-fluorenylmethoxycarbonyl), HOBt (1-hydroxybenzotriazole), K_2CO_3 (potassium carbonate), $MgSO_4$ (magnesium sulfate), MsCl (methylsulfonyl chloride), MTBE (*tert*-butyl methyl ether), NaH (sodium hydride), $NaHCO_3$ (sodium bicarbonate), nBuLi (n-butyllithium), PCC (pyridinium chlorochromate), PetEther (petroleum ether), QCl (tetrabutylammonium chloride), rt (room temperature), TBTU (*O*-benzotriazolyl-

N,N,N',N'-tetramethyluronium-tetrafluoroborate), TEA (triethyl amine), TFA (trifluoroacetic acid), THF (tetrahydrofuran), TMOF (trimethylorthoformate), TMAD (*N,N,N',N'*-tetramethylazodicarboxamide), TosCl (toluenesulfonyl chlorid

The following list of compounds were synthesized according to the below mentioned methods:

(5Z)-5-(4-hydroxy-benzilidene)-thiazolidine-2, 4-dione

(5Z)-5-(3-methoxy-benzilidene)-thiazolidine-2,4-dione

(5Z)-5-(1,3-benzodioxol-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5E)-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one

(5Z)-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-(2,3-dihydro-1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5E)-5-[(7-methoxy-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(9,10-dioxo-9,10-dihydroanthracen-2-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(2,2-difluoro-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-(1,3-dihydro-2-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-(1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-[(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(4-methyl-3,4-dihydro-2H-1,4-benzoxazin-7-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-(1,3-benzodioxol-5-ylmethylene)-2-imino-1,3-thiazolidin-4-one

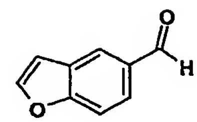
The following intermediate aldehydes are commercially available: 2,2-Difluoro-1,3-benzodioxole-5-carboxaldehyde, 1,3-Benzodioxole-5-carboxaldehyde, 1,4-Benzodioxan-6-carboxaldehyde, 9,10-Dioxo-9,10-dihydro-anthracene-2-carbaldehyde, 2,3-Dihydro-benzo[b]furan-5-carboxaldehyde, 3-Methoxy-4,5-methylenedioxybenzaldehyde.

Thiazolidinedione and Rhodanine are commercially available as well. Intermediate aldehydes, 5-Formyl-1-benzofuran, 4-Methyl-3-oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbaldehyde, 4-Methyl-3,4-dihydro-2H-benzo[1,4]oxazine-7-carbaldehyde and 1,3-Dihydroisobenzofuran-5-carbaldehyde, were synthesized according to the protocols as mentioned below.

The HPLC, NMR and MS data provided in the examples described below were obtained as followed: HPLC: column Waters Symmetry C8 50 x 4.6 mm, Conditions: MeCN/H_2O, 5 to 100% (8 min), max plot 230-400 nm; Mass spectra: PE-SCIEX API 150 EX (APCI and ESI), LC/MS spectra: Waters ZMD (ES); ^{1}H-NMR: Bruker DPX-300MHz.

The purifications were obtained as followed: Preparative HPLC Waters Prep LC 4000 System equipped with columns Prep Nova-Pak®HR C186 µm 60Å, 40x30mm (up to 100mg) or 40x300 mm (up to 1g). All the purifications were performed with a gradient of MeCN/H_2O 0.09% TFA.

Intermediate 1: Preparation of 5-formyl-1-benzofuran



Step I

Ethyl-2-formyl-4-bromophenoxy acetate:

A mixture of 5-bromosalicylaldehyde (50g, 0.248mol), ethylbromoacetate (42g, 0.248mol) and K_2CO_3 (68g, 0.49mol) in dry DMF (200mL) was stirred at RT for 12h. The reaction mixture was filtered and filtrate diluted with water. The mixture was extracted with diethylether (4x200mL), washed with brine and concentrated to give crude ethyl-2-formyl-4-bromophenoxy acetate (64g, 90%) as a solid.

Step II

4-Bromo-2-formylphenoxy acetic acid:

A mixture of ethyl-2-formyl-4-bromophenoxy acetate (60g, 0.209mol), LiOH (7.5g, 0.31mol), THF (250mL) and water (100mL) was stirred at RT for 24h. The reaction mixture was concentrated under reduce pressure and residue acidified with 1.5N HCl to pH=2. The solid precipitate obtained was filtered and dried to give 4-bromo-2-formyl-phenoxy acetic acid (50g, 94%).

Step III

5-Bromo-1-benzofuran:

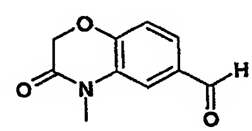
To a mixture of 2-formyl-4-bromophenoxy acetic acid (50g, 0.192mol), sodium acetate (100g, 1.21mol) in acetic acid (250mL) at 100°C was added acetic anhydride (100mL) portions during a period of 3h. The reaction mixture was then refluxed for 20h. The solvent was removed by distillation and residue diluted with 3N HCl (500mL) and refluxed for 2h. The reaction mixture was then concentrated under vacuum and product extracted with pet. ether (3x200mL). The organic layer was washed with 10% $NaHCO_3$ solution and evaporated to give 5-bromo-1-benzofuran (15g, 40%) as a pale yellow liquid.

Step IV

5-Formyl-1-benzofuran (P1a in scheme 1 for example 9):

A mixture of 5-bromo-1-benzofuran (0.5g), Mg (0.92g, 0.038mol), I_2 (1 crystal) in dry THF (2.5mL) under N_2 atmosphere was refluxed for 30min. To this was added a solution of 5-bromo-1-benzofuran (4.5g) in 25mL of dry THF) as soon as the I_2 color disappear and refluxed for another 2h. The reaction mixture was then cooled to −40°C and added dry DMF (3.6g) drop-wise and slowly warmed to RT for a period of 12h. The reaction mixture was then cooled to 0°C and acidified with 3N HCl to pH=2 and stirred for 30min. The reaction mixture was then diluted with water (500mL), extracted with ethylacetate (2x200mL), washed with brine and dried. The solvent was removed under vacuum and purified by column chromatography over silica gel (pet. ether/CH_2Cl_2) to give 5-formyl-1-benzofuran (2g, 54%) as a liquid. LC-MS: M/Z ESI: 1.47 min, 147.34 (M+1).

Intermediate 2: Preparation of 4-Methyl-3-oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbaldehyde



Step I

2-(N-methylamino)-phenol:

1g of benzoxazole was dissolved in 20 ml of THF. 0.9g of $NaBH_4$ were added under nitrogen and stirring. The suspension was cooled to 0°C and 0.86 ml of acetic acid dissolved in 5ml THF were slowly added, keeping the reaction temperature below 5°C. The reaction was stirred at 0°C for 30 minutes and for further 12 hours at room temperature. The reaction mixture was again cooled to 0°C and 50ml of sat. NH_4Cl solution were added carefully. The phases were separated and the aqueous layer extracted twice with EtOAc. The combined organic layers were washed with brine, dried over MgSO4 and filtered. Removal of the solvent afforded 0.97g (of pure 2-(N-methylamino)-phenol.

Step II

4-Methyl-4H-benzo[1,4]oxazin-3-one

1g of 2-(N-methylamino)-phenol were dissolved in chloroform, followed by the addition of 10ml of sat. $NaHCO_3$ in water. To this suspension was added slowly under vigorous stirring a solution of 1g of 2-chloroacetylchloride in acetone. The reaction mixture was stirred for 2 hours at room temperature. The layers were separated. The organic layer was washed with water and dried over Na_2SO_4. After evaporating the solvent, the red oil was taken up in 30 ml DMF and 1g of K_2CO_3 were added and the slurry was heated at 70°C for additional 2 hours. The cyclization was followed by TLC. 200 ml of EtOAc were added and the organic layer was washed 3x with 0.1N HCl and 5x with brine. The remaining organic layer was dried over MgSO4 and filtrated. EtOAc was removed under reduced pressure affording 1.45g of pure 4-methyl-4H-benzo[1,4]oxazin-3-one.

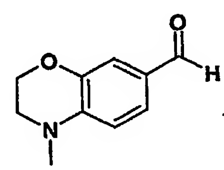
Step III

4-Methyl-3-oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbaldehyde (compound P1a of scheme 2, for use in the preparation of the compound of example 10 below)

1g of $AlCl_3$ were suspended in 10 ml DCM, 0.5 ml of nitromethane were added to dissolve $AlCl_3$, and the solution was cooled to 0°C. 4-Methyl-4H-benzo[1,4]oxazin-3-one (0.5g, 3.06 mmol) dissolved in DCM was added to the above solution and stirred for 15 minutes at 0°C. To this solution was further added 0.36ml of bis-chloromethyl-methylether in DCM. The reaction was stirred at 0°C for 15 minutes and at room temperature for 3h. The crude reaction mixture was then poured onto ice, the layers were separated and the organic phase was washed with $NaHCO_3$ and brine. After drying over $MgSO_4$ and filtration the solvent was evaporated, which afforded 0.43g of crude product. The dark oil was purified by flash chromatography using EtOAc and cyclohexane as eluents, affording 0.2g (37%) of 4-methyl-3-oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbaldehyde as colourless solid.

HPLC: 2.07 min. LC-MS: M/Z ESI: 1.31 min, 192.28 (M+1).

Intermediate 3: Preparation of 4-methyl-3,4-dihydro-2H-benzo[1,4]oxazine-7-carbaldehyde



Step I

4-Methyl-3,4-dihydro-2H-benzo[1,4]oxazine, 0.97g of 2-(N-methylamino)-phenol were dissolved in 50ml acetone, followed by the addition of 2g of K_2CO_3 dissolved in water. To this suspension was added slowly a solution of 2.66g of dibromoethane in acetone. The reaction mixture was stirred for 22 hours under reflux. Acetone was evaporated and 200ml of EtOAc were added and the organic layer was washed 3x with 0.1N HCl and 3x with brine. The remaining organic layer was dried over MgSO4 and filtrated. EtOAc was removed under reduced pressure affording 1g of pure 4-methyl-3,4-dihydro-2H-benzo[1,4]oxazine.

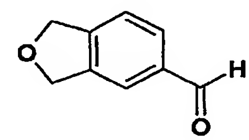
Step II

4-Methyl-3,4-dihydro-2H-benzo[1,4]oxazine-7-carbaldehyde (compound P1a of scheme 2, for use in the preparation of the compound of example 11 below).

4-Methyl-3,4-dihydro-2H-benzo[1,4]oxazine was dissolved in 200ul DMF under Argon. $POCl_3$ was added under Argon. The reaction was heated and a closed vial at 90°C for 75min. 1ml of NaAc in water was added and stirred while a brown oil was formed. The oil was extracted with DCM. The organic layer was washed with brine, dried and evaporated to dryness, affording 0.18g (76%) of 4-Methyl-3,4-dihydro-2H-benzo[1,4]oxazine-7-carbaldehyde as colourless solid.

LC-MS: M/Z ESI: 1.37 min, 178.35 (M+1).

Intermediate 4: Preparation of 1,3-Dihydroisobenzofuran-5-carbaldehyde



Step I

(1,3-Dihydro-isobenzofuran-5-yl)-methanol

In a round bottom flask with reflux condenser were placed 1.0g of 3-Prop-2-ynyloxy-propyne and 2.08g of propargylic alcohol in 10ml ethanol, followed by the addition of 9.8mg of tris(triphenylphosphine)rhodium chloride (Wilkinson catalyst) at room temperature. The reaction was heated up to 70°C, while the reaction colour turned yellow rapidly. After 1 day stirring at r.t., TLC analysis showed complete conversion of the starting material. The solvent was evaporated, diluted with DCM and extracted with H_2O, dried over MgSO4. The brown mixture was purified by flash chromatography using 8/2 cyclohexane / AcOEt as mobile phase affording (1,3-dihydro-isobenzofuran-5-yl)-methanol as a colourless pure solid (0.92g, 60%).

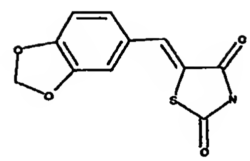
Step II

1,3-Dihydroisobenzofuran-5-carbaldehyde (compound P1a of scheme 2, for use in the preparation of the compound of example 8 below)

(1,3-Dihydro-isobenzofuran-5-yl)-methanol (440mg, 2.9mmol) was dissolved in 20 ml of DCM. 1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (Dess-Martin reagent) (1.3g, 3.2mmol) was added and the reaction was stirred at r.t. for 4h. The reaction mixture was diluted with ether and extracted 2x with NaOH 1N, 2x with H_2O and dried over $MgSO_4$. The crude product was sufficiently pure and used without any further purification. HPLC: 2.00 min. LC-MS: M/Z ESI: 1.50 min, 149.18 (M+1).

Examples

Example 1: Preparation of (5Z)-5-(1,3-benzodioxol-5-ylmethylene)-1,3-thiazolidine-2,4-dione



In a 100ml round bottom flask were placed 3.9g of thiazolidine, 5g of piperonal and 1.65ml of piperidine in 50ml of DME. The reaction was stirred for 3h at 120°C and then slowly cooled to room temperature, while the desired condensation product crystallized. The crystals were filtered, washed with DME (rt.) and than recrystallized from DME (25ml), affording 3.2g of pure (5Z)-5-(1,3-benzodioxol-5-ylmethylene)-1,3-thiazolidine-2,4-dione. The corresponding potassium salt was obtained via the following route: (5Z)-5-(1,3-benzodioxol-5-ylmethylene)-1,3-thiazolidine-2,4-dione was suspended in THF, followed by the addition of 1N solution of KOH in water (1.0 eq.). A clear solution has been obtained, which upon lyophilization gave pure potassium salt of (5Z)-5-(1,3-benzodioxol-5-ylmethylene)-1,3-thiazolidine-2,4-dione.

HPLC: 3.48 min. LC-MS: M/Z

ESI: 1.31 min, 248.12 (M-1). NMR (parent): ^1H NMR (DMSO-d6) δ 12.5 (br. s, 1H), 7.71 (s, 1H), 7.06-7.16 (m, 3H), 6.12 (s, 2H).

In cases were the final compounds did not crystallize from the reaction solutions, small quantities of water were added, leading to precipitating the desired condensation product.

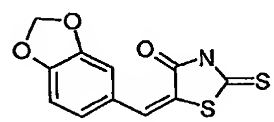
The crude was either recrystallized from an appropriate solvent like DME. Methanol, EtOAc or purified by flash-chromatography using EtOAc, cyclohexane mixtures as eluents.

Alternatively the final compounds could be synthesized in a parallel manner according to the following protocol:

In a parallel synthesizer Quest 210™ was placed the corresponding aldehyde, to which was added a mixture of piperidine (17.9 mg/tube) and 2,4-thiazolidinedione (49.2 mg/tube) in

DME (2ml/tube). The reactions were stirred for 3h at 120°C and then cooled to room temperature under agitation. 2ml of H_2O were added. Those compounds, which precipitated were filtered off via the lower manifold. The remaining clear solutions were reduced in volume, followed by the addition of water. The so formed solids were filtered and washed with little amount of DME, affording pure condensation products.

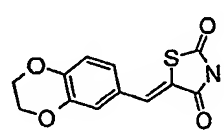
Example 2: Preparation of (5E)-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one



In a 24ml vial was placed 1g of commercially available rhodanine, 1.3g of piperonal and 0.5ml of TEA in 10ml of DME. The reaction was stirred for 5h at 120°C and then cooled to room temperature upon which the final product precipitated. The solid was filtered and washed with DME affording 1.6 g (80%)of orange powder.

LC-MS: M/Z ESI: 1.46 min, 266.00 (M+1), 264.08 (M-1). NMR (parent): ^{1}H NMR (DMSO-d6) δ 13.75 (br. s, 1H), 7.58 (s, 1H), 7.08-7.18 (m, 3H), 6.14 (s, 2H).

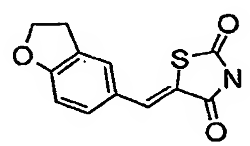
Example 3: Preparation of (5Z)-5-(2,3-dihydro-1,4-benzodioxin-6-yl-methylene)-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from 2,3-dihydro-1,4-benzodioxin-6-carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.

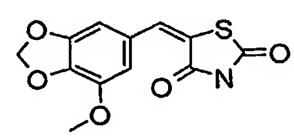
264 (M+1), 262 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.52 (br. s, 1H), 7.68 (s, 1H,), 7.09 (dd, 2H, J= 1.9, 7.1), 7.00 (d, 1H, J= 9.0Hz), 4.36-4.22 (m, 4H).

Example 4: Preparation of (5Z)-5-(2,3-dihydro-1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione



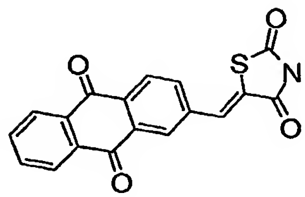
Following the general method as outlined in Example 1, starting from 2,3-dihydro-1-benzofuran-5-carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.
248 (M+1), 246 (M-1). ^{1}H NMR: (DMSO-d6) δ 9.80 (br. s, 1H), 7.37 (s, 1H,), 7.25 (d, 1H, J = 8.3), 7.21 (s, 1H), 6.80 (d, 1H, J = 8.3Hz), 4.54 (t, 2H, J = 8.85), 3.19 (t, 2H, J = 8.85)

Example 5: Preparation of (5E)-5-[(7-methoxy-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione



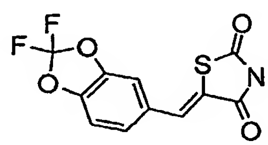
Following the general method as outlined in Example 1, starting from 7-methoxy-1,3-benzodioxol-5-yl)carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.
280 (M+1), 278 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.63 (br. s, 1H), 7.78 (s, 1H,), 7.65 (s, 1H), 7.57 (d, 1H, J= 8.5Hz), 7.45 (dd, 2H, J= 0.8, 7.6).

Example 6: Preparation of (5Z)-5-[(9,10-dioxo-9,10-dihydroanthracen-2-yl)methylene]-1,3-thiazolidine-2,4-dione



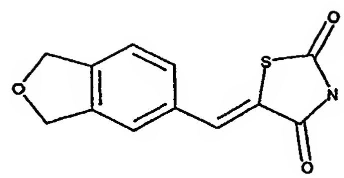
Following the general method as outlined in Example 1, starting from (9,10-dioxo-9,10-dihydroanthracen-2-yl)carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.
336 (M+1), 334 (M-1).

Example 7: Preparation of (5Z)-5-[(2,2-difluoro-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from (2,2-difluoro-1,3-benzodioxol-5-yl)carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.
286 (M+1), 284 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.63 (br. s, 1H), 7.78 (s, 1H,), 7.65 (s, 1H), 7.57 (d, 1H, J= 8.5Hz), 7.45 (dd, 2H, J= 0.8, 7.6)

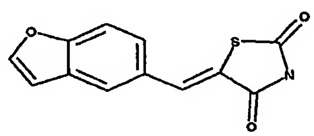
Example 8: Preparation of (5Z)-5-(1,3-dihydro-2-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from 1,3-dihydro-2-benzofuran-5-carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.

248 (M+1), 246 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.60 (br. s, 1H), 7.80 (s, 1H,), 7.56-7.42 (m, 2H), 5.03 (s, 4H)

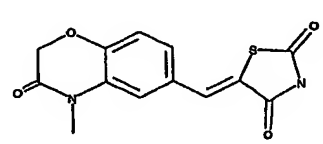
Example 9: Preparation of (5Z)-5-(1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from 1-benzofuran-5-carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.

264 (M+1), 244 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.58 (br. s, 1H), 8.10 (d, 1H, J = 2.2Hz), 7.92 (s, 2H), 7.74 (d, 1H, J = 8.6Hz), 7.57 (d, 1H, J = 8.6Hz), 7.07 (s, 1H)

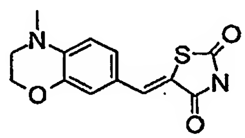
Example 10: Preparation of (5Z)-5-[(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)methylene]-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from [(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)carbaldehyde and 1,3-thiazolidine-2,4-dione, the title compound was obtained.

291 (M+1), 289 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.58 (br. s, 1H), 7.81 (s, 1H), 7.41 (s, 1H), 7.13-7.26 (d, 2H), 4.74 (s, 2H), 2.99 (s, 3H)

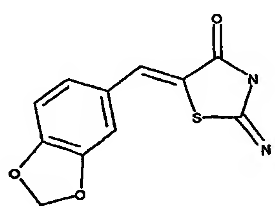
Example 11: Preparation of (5Z)-5-[(4-methyl-3,4-dihydro-2H-1,4-benzoxazin-7-yl)methylene]-1,3-thiazolidine-2,4-dione



Following the general method as outlined in Example 1, starting from [(4-methyl-3,4-dihydro-2H-1,4-benzoxazin-7-yl)methylene and 1,3-thiazolidine-2,4-dione , the title compound was obtained.

277 (M+1), 275 (M-1). ^{1}H NMR: (DMSO-d6) δ 12.34 (br. s, 1H), 7.60 (s, 1H), 7.08 (d, 1H, J = 8.5Hz), 6.88 (s, 1H), 6.79 (d, 1H, J = 8.5Hz), 4.21 (m, 2H), 3.41 (m, 2H), 2.94 (s, 3H).

Example 12: Preparation of (5Z)-5-(1,3-benzodioxol-5-ylmethylene)-2-imino-1,3-thiazolidin-4-one



Following the general method as outlined in Example 1, starting from 1,3-benzodioxol-5-carbaldehyde and 2-imino-1,3-thiazolidin-4-one, the title compound was obtained.

249 (M+1), 247 (M-1). ^{1}H NMR: (DMSO-d6)

Example 13 : Preparation of a pharmaceutical formulation

The following formulation examples illustrate representative pharmaceutical compositions according to the present invention being not restricted thereto.

Formulation 1 – Tablets

A compound of formula (I) is admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ration. A minor amount of magnesium stearate is added as a lubricant. The mixture is formed into 240-270 mg tablets (80-90 mg) of active azolidinone compound per tablet) in a tablet press.

Formulation 2 – Capsules

A compound of formula (I) is admixed as a dry powder with a starch diluent in an approximate 1:1 weight ratio. The mixture is filled into 250 mg capsules (125 mg of active azolidinone compound per capsule).

Formulation 3 – Liquid

A compound of formula (I) (1250 mg), sucrose (1.75 g) and xanthan gum (4 mg) are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously prepared solution of microcrystalline cellulose and sodium carboxymethyl cellulose (11:89, 50 mg) in water. Sodium benzoate (10 mg), flavor, and color are diluted with water and added with stirring. Sufficient water is then added to produce a total volume of 5 mL.

Formulation 4 – Tablets

A compound of formula (I) is admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ratio. A minor amount of magnesium stearate is added as a lubricant. The mixture is formed into 450-900 mg tablets (150-300 mg of active azolidinone compound) in a tablet press.

Formulation 5 – Injection

A compound of formula (I) is dissolved in a buffered sterile saline injectable aqueous medium to a concentration of approximately 5 mg/ml.

Example 14 : Biological assays

A. a) High Throughput PI3K lipid kinase assay (binding assay):

The assay combines the scintillation proximity assay technology (SPA, Amersham) with the capacity of neomycin (a polycationic antibiotic) to bind phospholipids with high affinity and specificity. The Scintillation Proximity Assay is based on the properties of weakly emitting isotopes (such as ^{3}H, ^{125}I, ^{33}P). Coating SPA beads with neomycin allows the detection of phosphorylated lipid substrates after incubation with recombinant PI3K and radioactive ATP in the same well, by capturing the radioactive phospholipids to the SPA beads through their specific binding to neomycin.

To a 384 wells MTP containing 5 µl of a chemical compound library (containing 6% DMSO), the following assay components are added. 1) 5 µl (58 ng) of Human recombinant GST-PI3Kγ (in Hepes 40 mM, pH 7.4, DTT 1 mM and ethylenglycol 5%) 2) 10 µl of lipid micelles and 3) 10 µl of Kinase buffer ([^{33}P]γ–ATP 45µM/60nCi, $MgCl_2$ 30mM, DTT 1mM, β–Glycerophosphate 1mM, $NaVO_4$ 100 µM, Na Cholate 0.3 %, in Hepes 40 mM, pH 7.4). After incubation at room temperature for 180 minutes, with gentle agitation, the reaction is stopped by addition of 60 µl of a solution containing 100 µg of neomycin-coated PVT SPA beads in PBS containing ATP 10mM and EDTA 5mM. The assay is further incubated at room temperature for 60 minutes with gentle agitation to allow binding of phospholipids to neomycin-SPA beads. After precipitation of the neomycin-coated PVT SPA beads for 5 minutes at 1500 x g, radioactive *PtdIns(3)P* is quantified by scintillation counting in a Wallac MicroBeta ™ plate counter.

The values indicated in respect of PI3K γ refer to the IC_{50} (μM), i.e. the amount necessary to achieve 50% inhibition of said target. Said values show a considerable potency of the azolidinone-vinyl fused-benzene compounds with regard to PI3Kγ.

The tested compounds according to formula (I) display an inhibition (IC_{50}) with regard to PI3Kγ of less than 2 μM, more preferred equal or less than 1 μM.

Examples of inhibitory activities for test compounds of examples 1, 2 and 10 as set out in Table 1.

Example No	*PI3Kγ, IC_{50} (μM)*
1	0.05
2	0.06
10	0.03

Table 1: IC_{50} values of azolidinone-vinyl fused-benzene derivatives against PI3Kγ.

b) Cell based ELISA to monitor PI3K inhibition:

Measurement of Akt/PKB phosphorylation in macrophages after stimulation with C5a: Raw 264: Raw 264-7 macrophages (cultured in DMEM-F12 medium containing 10% Fetal Calf serum and antibiotics) are plated at 20'000 cells/well in a 96 MTP 24 h before cell stimulation. Previous to the stimulation with 50 nM of Complement 5a during 5 minutes, Cells are serum starved for 2h, and pretreated with inhibitors for 20 minutes. After stimulation cells are fixed in 4% formaldehyde for 20 minutes and washed 3 times in PBS containing 1% Triton X-100 (PBS/Triton). Endogenous peroxidase is blocked by a 20 minutes incubation in 0.6% H_2O_2 and 0.1% Sodium Azide in PBS/Triton and washed 3 times in PBS/Triton. Cells are then blocked by 60 minutes incubation with 10% fetal calf serum in PBS/Triton. Next, phosphorylated Akt/PKB is detected by an overnight incubation at 4°C with first antibody (anti phospho Serine 473 Akt IHC, Cell Signaling) diluted 800-fold in PBS/Triton, containing 5% bovine serum albumin (BSA). After 3

washes in PBS/Triton, cells are incubated for 60 minutes with a peroxidase conjugated goat-anti-rabbit antibody (1/400 dilution in PBS/Triton, containing 5% BSA), washed 3 times in PBS/Triton, and 2 times in PBS and further incubated in 100 µl of substrate reagent solution (R&D) for 20 minutes. The reaction is stopped by addition of 50 µl of 1 M SO_4H_2 and absorbance is read at 450 nm.

The values indicated reflect the percentage of inhibition of AKT phoshorylation as compared to basal level. Said values show a clear effect of the azolidinone-vinyl fused-benzene compounds on the activation of AKT phosphorylation in macrophages.

Compounds of examples 9 and 10, when used at 10µM completely inhibit C5a-mediated AKT phosophorylation. Examples 1, 2 or 4, when used at 10 µM, inhibit 80% of the C5a-mediated AKT-phosphorylation.

B. *In vitro* experiments:

In the experiments the following examples are based on, standard methods of in vitro fertilization have been used. With regard to the details of these methods, reference is made to the "WHO manual" (WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions, 4^{th} ed ition, Cambridge University Press 1999). In particular, the direct swim-up method can be taken from pp. 104 to 106 of this manual.

a) Effect of the PI3K inhibitor on the rapid motility of spermatozoa:

Spermatozoa are prepared according to the standard procedures of IVF. Briefly, spermatozoa are prepared from 3 oligoasthenospermic subjects undergoing semen analysis for couple infertility after informed consent. 10 µM of the tested compound of formula (I) are added directly to the seminal liquid and incubated for 2 hours for two hours at 37°C and 5% CO_2. The motility of the spermatozoa is then blindly evaluated under the microscope according to WHO manual procedures.

In a group of 7 samples taken from seven individuals, the tested phosphatidylinositol-3-kinase inhibitor is added in a higher concentration (100 µM). After the incubation with the

compound for two hours, swim-up selection of the spermatozoa is performed according to procedures described in the WHO-manual. The incubation of the sperm cells with a ten times higher concentration of the compound of formula (I) (100 μM) in combination with the swim-up selection results in a significant increase of progressive motility in all of the seven samples.

Results may be obtained in a similar experiment on samples from higher numbers of patients. The sperm cells are submitted to the swim-up selection method. Treatment with 10μM of the tested phosphatidylinositol-3-kinase inhibitor results in an increase in the progressive motility as compared to the control (patients without LY294002). Treatment of samples from patients with 100μM of the inhibitor results in an increase of the motility as compared to the control.

The effect of 100μM of the compound of formula (I) on the viability of the spermatozoa is also assessed. The incubation with the tested PI3K inhibitor is carried out to observe the alteration of the vitality of the cells for two hours and after 48 hours.

Further experiment may be carried out in the same manner as outlined above on samples from 12 individual patients.

b) Effect of example 1 compound on further sperm cell parameters:

The increase in forward motility, demonstrated in the above mentioned part A, is associated with an increase in sperm parameters related to fertilization activity of the spermatozoa *in vitro*, such as percentage curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL) and hyper-activated sperm fraction (HA). These parameters are determined by computer aided sperm analysis (CASA) in sperm samples from different oligo-asthenospermic subjects. Each of these parameters are increased in a statistically significant manner by incubation with 10 μM of compound of example 1 as compared to the control sample, indicating a significant overall improvement of sperm cell fertilization activity.

c) Effect of the tested compound of formula (I) on forward motility of H_2O_2 or LiCl treated spermatozoa:

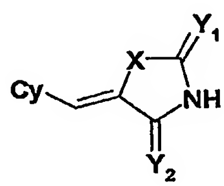
It is well known that reactive oxygen species (ROS), which may be generated during sperm preparation for IVF, exert detrimental effects on sperm fertilization potential. In particular, among ROS, H_2O_2 strongly reduces motility when added to sperm samples at micromolar concentrations. Therefore, the effect of the tested compound of formula (I) on H_2O_2 treated sperm cells is evaluated. The compound is added to swim-up selected spermatozoa samples from oligo-asthenospermic patients in amounts of 10 μM either alone or in combination with 200 μM of H_2O_2.

LiCl is known as having inhibition properties on sperm cell motility. An incubation of swim-up selected spermatozoa with 10 μM of tested compound of formula (I) for two hours either with or without different concentrations of LiCl results in reversing the effect of LiCl induced inhibition of sperm motility.

In this example, the activity of compounds of formula (I) to rescue spermatozoa from deleterious agents, which may be generated in assisted fertilization techniques has been demonstrated. Therefore, the invention provides for a major improvement of ART, leading to a higher fertilization rate and eliminating some of the most serious drawbacks of these techniques.

Claims

1. Process for improving the fertilization activity of spermatozoa, in particular for increasing spermatozoa motility, comprising the step of treating spermatozoa with a compound of formula (I)



(I)

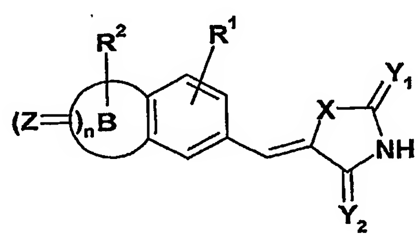
as well as its geometrical isomers, its optically active forms as enantiomers, diastereomers and its racemate forms, as well as pharmaceutically acceptable salts and pharmaceutically active derivatives thereof, wherein

X is S, O or NH;

Y^1 and Y^2 are independently S, O or –NH;

Cy is a 5 to 8 membered carbocyclic or heterocyclic group may be fused with an aryl, heteroaryl, cycloalkyl or heterocycloalkyl group.

2. Process according to claim 1, whereby the compound has formula (I')



(I')

wherein B is a 5-8 membered heterocyclic ring or a carbocyclic group, said carbocyclic group may be fused with an aryl, heteroaryl, cycloalkyl or heterocycloalkyl group;

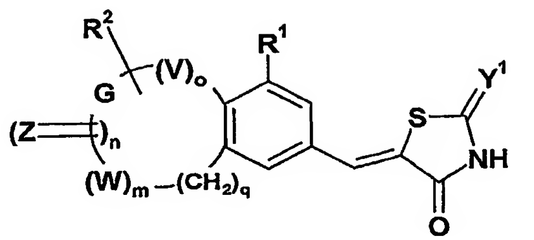
R^1 is H, CN, carboxy, acyl, C_1-C_6-alkoxy, halogen, hydroxy, acyloxy, C_1-C_6-alkyl carboxy, C_1-C_6-alkyl acyloxy, C_1-C_6-alkyl alkoxy, alkoxycarbonyl, C_1-C_6-alkyl alkoxycarbonyl, aminocarbonyl, C_1-C_6-alkyl aminocarbonyl, acylamino, C_1-C_6-alkyl acylamino, ureido, C_1-C_6-alkyl ureido, amino, C_1-C_6-alkyl amino, ammonium, sulfonyloxy, C_1-C_6-alkyl sulfonyloxy, sulfonyl, C_1-C_6-alkyl sulfonyl, sulfinyl, C_1-C_6-alkyl sulfinyl, sulfanyl, C_1-C_6-alkyl sulfanyl, sulfonylamino, C_1-C_6-alkyl sulfonylamino or carbamate;

R^2 is selected from the group comprising or consisting of H, halogen, acyl, amino, C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_1-C_6-alkyl carboxy, C_1-C_6-alkyl acyl, C_1-C_6-alkyl alkoxycarbonyl, C_1-C_6-alkyl aminocarbonyl, C_1-C_6-alkyl acyloxy, C_1-C_6-alkyl acylamino, C_1-C_6-alkyl ureido, C_1-C_6-alkyl amino, C_1-C_6-alkyl alkoxy, C_1-C_6-alkyl sulfanyl, C_1-C_6-alkyl sulfinyl, C_1-C_6-alkyl sulfonyl, C_1-C_6-alkyl sulfonylaminoaryl, aryl, heteroaryl, C_3-C_8-cycloalkyl or heterocycloalkyl, C_1-C_6-alkyl aryl, C_1-C_6-alkyl heteroaryl, C_2-C_6-alkenyl-aryl or -heteroaryl, C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, cyano, hydroxy, C_1-C_6-alkoxy, nitro, acylamino, ureido, sulfonylamino, sulfanyl, or sulfonyl;

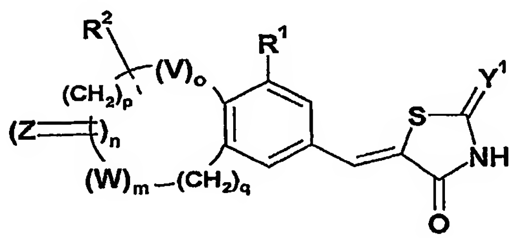
Z is O or S; n is 0, 1 or 2; and

X, Y^1, Y^2 are as above-defined.

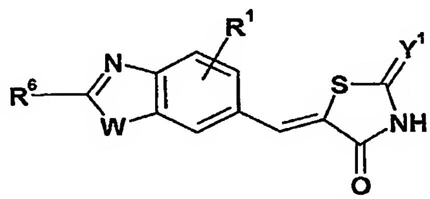
3. Process according to claim 1 or 2, wherein the compound is selected from any of formulae (Ia), (Ib), (Ic) or (Id)



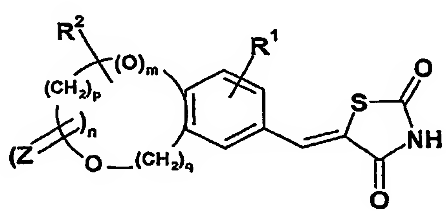
(Ia)



(Ib)



(Ic)

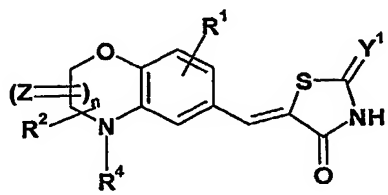


(Id)

wherein R^1, R^2, Y^1, Z and n are as above-defined, G is a C_1-C_5 alkylene or a C_1-C_5 alkenylene group, W and V are each independently from each other selected from O, S, $-NR^3$ wherein R^3 is H or a C_1-C_6 alkyl group, R^6 is H or OH, m, n and o are each

independently from each other 0 or 1, p and q are independently from each other an integer from 1 to 4.

4. Process according to any of the preceding claims, wherein the compound has formula (II)

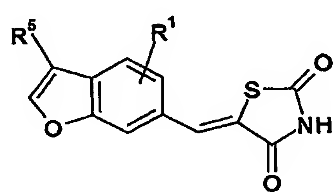


(II)

wherein Z, Y^1, R^1, R^2 are as above defined; n is 0 or 1;

R^4 is selected in the group comprising or consisting of H, acyl, C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_1-C_6-alkyl carboxy, C_1-C_6-alkyl acyl, C_1-C_6-alkyl alkoxycarbonyl, C_1-C_6-alkyl aminocarbonyl, C_1-C_6-alkyl acyloxy, C_1-C_6-alkyl, acylamino, C_1-C_6-alkyl ureido, C_1-C_6-alkyl amino, C_1-C_6-alkyl alkoxy or C_1-C_6-alkyl sulfanyl, C_1-C_6-alkyl sulfinyl, C_1-C_6-alkyl sulfonyl, C_1-C_6-alkyl sulfonylaminoaryl aryl, heteroaryl, C_3-C_8-cycloalkyl or heterocycloalkyl, C_1-C_6-alkyl aryl, C_1-C_6-alkyl heteroaryl, C_2-C_6-alkenyl-aryl or -heteroaryl, C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, hydroxy, C_1-C_6-alkoxy, C_1-C_6 alkyl carbamate, sulfonylamino, sulfanyl or sulfonyl.

5. Process according to any of claims 1 to 3, wherein the compound has formula (III)



(III)

wherein R^1 is as above defined;

R^5 is selected in the group comprising or consisting of H, halogen, acyl, amino, C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_1-C_6-alkyl carboxyl, C_1-C_6-alkyl acyl, C_1-C_6-alkyl alkoxycarbonyl, C_1-C_6-alkyl aminocarbonyl, C_1-C_6-alkyl acyloxy, C_1-C_6-alkyl, acylamino, C_1-C_6-alkyl ureido, C_1-C_6-alkyl amino, C_1-C_6-alkyl alkoxy or C_1-C_6-alkyl sulfanyl, C_1-C_6-alkyl sulfinyl, C_1-C_6-alkyl sulfonyl, C_1-C_6-alkyl sulfonylaminoaryl, aryl, heteroaryl, C_3-C_8-cycloalkyl or heterocycloalkyl, C_1-C_6-alkyl aryl, C_1-C_6-alkyl heteroaryl, C_2-C_6-alkenyl-aryl or -heteroaryl, C_2-C_6-alkynyl aryl or -heteroaryl, carboxy, cyano, hydroxy, C_1-C_6-alkoxy, nitro, acylamino, C_1-C_6 alkyl carbamate, ureido, sulfonylamino, sulfanyl or sulfonyl.

6. Process according to any of claims 1 to 5, wherein treating the spermatozoa with the compound of formula (I) is performed on seminal liquid comprising the spermatozoa.

7. Process according to any of claims 1 to 6, further comprising separating the spermatozoa by spermatozoa separation methods used in assisted reproduction techniques.

8. Process according to claim 7, wherein separating the spermatozoa is performed by a method selected from the wash and spin method, the sedimentation method, the direct swim-up method, the pellet and swim-up method, the buoyant density gradient method.

9. Process according to claim 8, wherein separating the spermatozoa is performed by the direct swim-up method.

10. Process according to any of the preceding claims, wherein the process is performed on mammal spermatozoa, in particular on human spermatozoa.

11. Process according to any of the preceding claims, wherein the compound of formula (I) is selected from the group consisting of

(5Z)-5-(4-hydroxy-benzilidene)-thiazolidine-2,4-dione

(5Z)-5-(3-methoxy-benzilidene)-thiazolidine-2,4-dione

(5E)-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one

(5Z)-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-(2,3-dihydro-1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5E)-5-[(7-methoxy-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(9,10-dioxo-9,10-dihydroanthracen-2-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(2,2-difluoro-1,3-benzodioxol-5-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-(1,3-dihydro-2-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-(1-benzofuran-5-ylmethylene)-1,3-thiazolidine-2,4-dione

(5Z)-5-[(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)methylene]-1,3-thiazolidine-2,4-dione

(5Z)-5-[(4-methyl-3,4-dihydro-2H-1,4-benzoxazin-7-yl)methylene]-1,3-thiazolidine-

2,4-dione

(5Z)-5-(1,3-benzodioxol-5-ylmethylene)-2-imino-1,3 -thiazolidin-4-one

(5-(2H-benzo[d]1,3-dioxolen-5-ylmethylene)-1,3 thiazolidine-2,4-dione.

12. Process according to any of the preceding claims, wherein said spermatozoa are treated with an amount of a compound of formula (I) in the range of about 0.01 to 1000 µM, about 5 to 500 µM, or about 10 to 100 µM.

13. Process according to any of the preceding claims, wherein treating the spermatozoa with a compound of formula (I) comprises incubating the spermatozoa for a period of time in the range of about 30 minutes to 10 hours or about 1 to 8 hours or about 2 to 6 hours at a temperature of around 37°C.

14. Spermatozoa obtainable by the process according to any of claims 1 to 13.

15. Use of a compound according to any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) for improving the fertilization rate in assisted reproduction techniques.

16. Use according to claim 15, wherein the assisted reproduction techniques are selected from in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), or intra-uterine insemination (IUI).

17. Use of a compound according to any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) for the preparation of a pharmaceutical composition for the treatment of infertility, in particular male infertility.

18. Use of a compound according to any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) for the preparation of a pharmaceutical composition for improving spermatozoa fertilization activity, in particular for increasing spermatozoa motility.

19. Method of ART therapy, comprising treating spermatozoa with a compound of any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III) as above-defined.

20. Method according to claim 19, wherein said ART are selected from in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), or intra-uterine insemination (IUI).

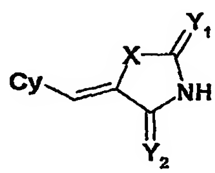
21. A medium for storage and/or transportation of spermatozoa comprising a compound of any of formulae (I), (I'), (Ia), (Ib), (Ic), (Id), (II) or (III).

22. Medium according to claim 21 for the storage and/or transportation of mammal spermatozoa, in particular human spermatozoa.

23. Medium according to any of claims 21 or 22, comprising an amount of a compound of formula (I) in the range of about 0.01 to 1000 µM, about 5 to 500 µM, or about 10 to 100 µM.

Abstract

The invention relates to a process for the improvement of spermatozoa fertilization activity, in particular for the increase of spermatozoa motility, by using a compound of formula (I).



(I)

The invention further relates to uses and methods of compounds of formula (I) in infertility and assisted reproduction techniques (ART) as well as to a medium for storage and/or transportation of spermatozoa comprising said PI3K inhibitors.

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